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Abstract

Multiple sclerosis (MS) is a progressive, neurodegenerative disease of the central nervous system characterized by widespread lesions and plaques that disrupt neural transmission. In addition to physical disability, cognitive impairment is experienced in about half of the MS population, which profoundly impacts vocational ability and quality of life. Amongst people with MS experiencing cognitive deficits, memory impairment is one of the most common symptoms. Assessing memory impairment in MS, a critical step in treatment, has been a difficult process. Traditional clinical batteries assessing memory impairment in MS may not adequately capture the multiple subprocesses of memory. Pattern separation, the ability to discriminate between similar yet distinct memories, is one aspect of memory that remains unexplored in the MS population. Previous research in animals and other memory-impaired populations links the underlying neuronal computational processes of pattern separation to the subsections of the hippocampus. Moreover, hippocampal atrophy is common in MS. Therefore, this study uses the Mnemonic Similarities Task, a behavioral measure of pattern separation, to investigate pattern separation performance in a sample of MS participants as well as its relationship to structural brain parameter of hippocampal atrophy and white matter microstructural integrity. Results revealed strong positive correlations whereby lower pattern separation performance was related to smaller hippocampal volumes. Microstructural analysis of white matter tracts revealed no differences between high and low MS pattern separation performers, although this may be due to sample size. Results have implications for clinical assessment and suggest a need for future research into how pattern separation ability is affected in MS patients.

PATTERN SEPARATION IN MS

MONTCLAIR STATE UNIVERSITY

Structural and Diffusion Parameters Related to Pattern Separation in Multiple Sclerosis

by

Mark Zuppichini

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Arts in General Psychology

May 2017

College of Humanities and Social Sciences Thesis Committee:

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PATTERN SEPARATION IN MS

STRUCTURAL AND DIFFUSION PARAMETERS RELATED TO PATTERN
SEPARATION IN MULTIPLE SCLEROSIS

A THESIS

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Montclair State University

Montclair, NJ

2017

Acknowledgements

I would like to thank Montclair State University for giving me the opportunity to learn and grow as a professional in my field of interest. Thank you to the research assistants of the Cognition & Neurocognitive Disorders Research Lab who helped code the data: Megan Groner, Petty Tineo, and Vanessa Gomez. Thank you Dr. Lowe and Dr. Pehrson for providing me with excellent help and advice. A huge thank you to Dr. Joshua Sandry, who I consider my greatest teacher thus far. This study was funded in part by a pilot research grant from the Foundation of the Consortium of Multiple Sclerosis Centers to Joshua Sandry. Lastly, thank you to my family, for I could not have done this without your continuing love & support.

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Structural and Diffusion Parameters Related to Pattern Separation in Multiple Sclerosis

Introduction

Multiple sclerosis (MS) is a degenerative disease of the central nervous system affecting both white and gray matter (Sacco et al., 2015). The degeneration of neurons produces widespread lesions and plaques in the central nervous system, which can disrupt axonal transmission (Trapp, Ransohoff, & Rudick, 1999; Wang et al., 2015). Although the exact etiology of the disease is still under investigation, four clinical courses have been identified based on the rate the disease progresses (Herndon, 2002). Relapsing-remitting MS is defined by a disease course with periods of exacerbation and periods of almost full recovery of symptoms. About 80% of individuals with RRMS go on to develop secondary-progressive MS, which is characterized by progressive, and gradual exacerbation of symptoms with little or no remission (Lublin et al., 2014). Progressive-relapsing MS course exhibits progressive decline after the start of the disease with acute periods of relapse. Lastly, primary-progressive MS includes the gradual increase of symptoms from onset with no distinct relapsing or exacerbation periods (Herndon, 2002; Lublin et al., 2014).

Neurocognitive Impairment in MS

Along with physical disability, the disruption of neural transmission in people with MS can cause profound and wide-ranging cognitive impairments (Chiaravalloti & DeLuca, 2008; Rao, Leo, Bernardin, & Unverzagt, 1991). Indeed, cognitive impairments associated with MS are evident in cognitive domains related to attention, information processing speed, working memory, executive functioning, and long-term memory (Benedict, Cookfair, et al., 2006; Rocca et al., 2015). These cognitive deficits can have a

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profound impact on quality of life and employment prospects (Goverover, Strober, Chiaravalloti, & DeLuca, 2015; Strober et al., 2012). For example, neuropsychological evaluation was used to classify MS participants into cognitively impaired or cognitively unimpaired. The authors found no significant differences between the impaired and non-impaired groups with respect to physical disability or illness duration, but they did find that the participants in the cognitively impaired group were less likely to be working, less likely to attend social events, reported more sexual dysfunction, experienced greater difficulty in every-day tasks, and also showed more psychopathology than the cognitively unimpaired MS group (Rao, Leo, Ellington, et al., 1991). Notably, the cognitively impaired participants in this study consistently failed the selective-reminding task (Buschke, 1973), a verbal learning and memory task, providing evidence that memory is a commonly impaired cognitive domain.

Long-term memory, the ability to recall information later in time, is one of the most consistently impaired cognitive functions in MS with up to 65% of patients showing impairment (Chiaravalloti & DeLuca, 2008; Rao et al., 1993; Rao, Leo, Bernardin, et al., 1991; Rocca et al., 2015). Given the overlap between cognitive impairment and quality of life, there is a need to better understand cognitive impairment generally, and memory impairment in particular, to potentially increase patients' quality of life. In order to understand memory impairment in MS it is important to first understand how memory has historically been assessed in MS.

Memory Assessments in Neuropsychological Batteries. Assessing memory impairment in MS is a difficult process. Major issues with assessment include but are not limited to inconsistent definitions of impairment, the use of different batteries to assess

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the same domains, and a lack of understanding and agreement about what cognitive domains are being assessed. This section will specifically review memory assessments in neuropsychological batteries commonly used in MS while highlighting some of the major issues that impact understanding memory problems in MS.

Inconsistent definitions and arbitrary cut offs add to the difficulty of measuring memory impairment in MS (Sandry, Akbar, Zuppichini, & DeLuca, 2016). For example, an early study operationalized impairment as MS participants who scored below the fifth percentile of the normal control participants on four or more tests within a neuropsychological battery (Rao, Leo, Bernardin, et al., 1991). This particular study consisted of 31 neuropsychological tests containing multiple tests of different aspects of memory. Therefore, it is possible that two MS participants classified as memory impaired may have failed two completely different memory tests and this does not tell us what about memory is affected by the MS disease progression.

Other studies that do not have explicit definitions of impairment operationalized impairment as whether the MS participants scored significantly lower than healthy controls (Staples & Lincoln, 1979) or whether MS participants showed “abnormal” scores on multiple tests (Rovaris et al., 2000). Many studies of MS-related memory impairment classify MS participants who scored 1.5 or 2 standard deviations below the corresponding normative mean in the respective memory tests as mnemonically impaired (González Torre et al., 2017; Heesen et al., 2010; Swirsky-Sacchetti et al., 1992). In the future, specific memory domains should have clear definitions for impairment linked to specific tests with specific cutoffs supported by research. The tests used to assess memory impairment also play a critical role in its definition.

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One of the first widely used batteries, termed the brief repeatable battery (BRB), was designed from a selection of tests that MS participants consistently failed (Rao, Leo, Bernardin, et al., 1991). Of the tests in the BRB, two are memory-related: one test assesses verbal learning and memory, the selective reminding test (SRT) (Buschke, 1973) and another assesses spatial memory, the spatial recall test (Rao, Leo, Bernardin, et al., 1991). The SRT assesses several components of learning and memory and involves the aural presentation of a list of 10 words, which the participant is then prompted to recall (Buschke, 1973; Buschke & Fuld, 1974). The participant is then selectively reminded of the words that were not recalled on the immediately preceding trial, and again told to recall as many of the words as possible. This continues until the participant can recall the entire list twice in a row or until the participant reaches six attempts, making the trials to criterion the dependent measure. The spatial recall test involves the 10-second presentation of a 4 x 6-inch checkerboard grid with seven black dots placed in random order on the grid. The participant is then given a blank grid with black checkers and asked to place the checkers on the grid to reproduce the pattern shown previously (Barbizet & Cany, 1967; Rao, Hammeke, McQuillen, Khatri, & Lloyd, 1984). While these two tests may be able to tell us something about verbal learning and memory, and spatial recall, the battery misses other aspects of memory. For example, the SRT does not assess recognition memory and neither test in this battery is able to assess any working memory components. Because these tests do not accurately assess all aspects of memory, the memory components of this battery may not be specific enough to identify what aspect of memory is impaired. Other batteries have also been developed to address the

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issue of which cognitive assessments most accurately and efficiently detect MS-related memory impairment.

The minimal assessment of cognitive function in multiple sclerosis (MACFIMS) was designed to assess impairment in the cognitive domains of processing speed, working memory, learning and memory, executive functions, visual perception/spatial processing, and language, and has shown to have good validity for the purpose of routine neuropsychological testing (Benedict, Cookfair, et al., 2006). The MACFIMS may be the most well-rounded battery but was chiefly designed to be sensitive to discriminate between patients with subtle deficits and healthy controls, monitor changes in cognitive functioning over time, aid in clinical decision-making, be applicable in multiple settings (languages, cultures, etc.), and to be parsimonious or quickly administered (Benedict et al., 2002). The parsimonious nature of the battery, therefore, does not allow for it to be a comprehensive assessment of cognitive functioning and results from this battery should not be viewed as such (Benedict et al., 2002).

The MACFIMS uses the California Verbal Learning Test second edition (CVLT-II) (Delis, Kramer, Kaplan, & Ober, 1987) as a verbal list learning assessment of memory and the Brief Visuospatial Memory Test—revised (BVMT-R) (Benedict, 1997) as a spatial memory test. The CVLT-II involves 16 words (that could be grouped into four categories, e.g. tools, fruits, clothing, etc.) being read aloud at 1-second intervals over five learning trials with the participant prompted to recall all of the words after each trial. An interference list is then presented that shares two of the categories presented in the original list and the participant is tested on this interference list. Immediately after the interference list recall test, the participant is asked to recall as many words from the

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original list as they can; participants are also tested on the original list 20 minutes later as a long-term measure. The test ends with a recognition test involving 44 words that the participant is asked to classify as from the original list or not. The BVMT-R involves the presentation of a sheet that has 6 figures on it for 10 seconds, then the participant is asked to draw the shapes exactly as they were and in the correct position on the page. This continues for a total of 3 trials and then the participant is tested again 20-25 minutes later, ending with a recognition trial that involves the presentation of 12 figures of which the participant has to classify as part of the original sheet or not. Both the CVLT and BVMT tap into the immediate free recall, delayed free recall, and recognition aspects of memory in their respective domains of verbal and visuospatial memory. The CVLT goes a step further by looking at how the interference of a somewhat similar list of words can affect memory, which the BVMT does not do. Together, these two tests are part of the most popular memory battery used today. However, as in the BRB, these two tests do mainly assess verbal and visuospatial memory and, although they may be good for clinical use, do not allow us to understand the underlying cognitive mechanisms of memory impairment.

Another battery, referred to as the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS), was developed with the purpose of creating a short assessment designed to capture the cognitive performance of MS patients and can be used by either clinical specialists or local healthcare workers (Langdon et al., 2012). This assessment was explicitly not designed to be either a full cognitive screen, but instead a brief monitoring instrument that could be internationally validated and standardized for global use (Langdon et al., 2012). To assess memory, the battery contains the same verbal

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and visuospatial tests of the MACFIMS, the CVLT and BVMT. However, the BICAMS only uses the immediate recall parts of these tests, and so assesses fewer aspects of memory than the MACFIMS.

One additional battery worth mentioning due to its frequent use in MS cognitive evaluation is the MS Functional Composite (MSFC) (Cutter et al., 1999). Created by the Clinical Outcomes Assessment Task Force for the purpose of evaluating arm function, leg function and ambulation, and cognitive function, the MSFC contains only one test of cognitive function, the Paced Auditory Serial Addition Test (PASAT), and no tests of memory (Cutter et al., 1999).

Another issue complicating the assessment of memory in MS concerns the clinical courses associated with the progression rate of the disease. Research shows that memory deficits and neuronal correlates significantly differ between the clinical courses (Sumowski et al., 2017). The upshot of which means two studies could be run with the exact same methods but present different results if their samples consisted of MS participants with differing clinical courses. Therefore, it is recommended to group together MS participants with the same clinical courses for comparison in research studies with more than one clinical course type in their MS sample.

Currently, ample difficulties diminish the ability to assess memory impairment in MS. It is necessary to create a standardized and consistently used definition of memory impairment for appropriate classification of people into memory impaired and unimpaired groups. This will allow for compatible comparisons across future research studies. A definition of memory impairment should set a clear, consistent, and evidence based cut-off score for each test that is used, and the memory test battery used should

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include tests that measure most, if not all, aspects of the cognitive functions underlying memory. Once these issues have been addressed, a more accurate assessment of memory impairment in MS can be researched. First it is important to begin to tease apart the underlying memory processes impaired in MS.

Nature of MS-related Memory Impairment. The nature of MS-related memory impairment is still under investigation and often the findings depend on the clinical tests used for investigation (see previous section). Studies support the notion that MS differentially affects various types of memory systems with some processes showing substantial impairment and others remaining mostly intact (Thornton & Raz, 1997). For example, short term memory is observed to be relatively intact (Rao, Leo, Haughton, Aubin-Faubert, & Bernardin, 1989) while working memory is impaired (Rao et al., 1993). This section will review some of the major findings regarding the nuanced nature of memory impairment in MS.

Early research on MS-related memory impairment suggested that a retrieval deficiency was largely responsible for the memory deficit. For example, researchers tested MS participants and demographically matched controls on a battery of memory assessments. The authors found that the MS group performed significantly worse than the control group on all tests except for tests of recognition memory (Rao et al., 1993). More importantly though, for their retrieval deficiency hypothesis, the largest difference between the MS and control groups were observed in the long-term retrieval measure from the SRT, total recall on the spatial recall test, and the free-recall (immediate and delayed) section of the story recall test (Rao et al., 1993). The crux of the retrieval deficit hypothesis relies on the fact that free recall scores show deficits but recognition scores do

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not. The reasoning here is that, compared to free recall, recognition imposes less of a demand on retrieval functions while still necessitating properly functioning encoding and storage processes (Thornton & Raz, 1997). Therefore, based on these score differences, it seems that MS participants are able to encode some of the information properly but cannot retrieve as much information as healthy controls.

Another study provided evidence for people with MS having recognition deficits (Beatty, Goodkin, Monson, Beatty, & Hertsgaard, 1988). Participants learned a list of 14 words, which they were tested on immediately for four trials, and after the four trials there was a 30-minute delay free recall followed by a recognition test. The MS participants scored worse on all three measures, providing evidence that MS did have recognition deficits. The authors also attributed their deficits on both immediate and delayed recall to poor encoding.

Other research suggests that MS participants recall less because they learn less initially, and therefore, acquisition is the main problem, not retrieval. Initial evidence for acquisition deficits came from a study where MS participants were given as many as 15 trials for the SRT (instead of the traditional six trials) and, although the MS group required more trials to learn the list than healthy controls, once the list was learned, it was recalled at similar rates to healthy controls (DeLuca, Barbieri-Berger, & Johnson, 1994).

A more recent study used both a list-learning experiment and meta-analysis to examine the retrieval and acquisition hypotheses (Lafosse, Mitchell, Corboy, & Filley, 2013). The authors used data from MS and healthy controls, of which about half took the CVLT and the other half took the CVLT-II. The authors reasoned that the scores showed equivalency and they aggregated both the CVLT and CVLT-II scores to increase

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statistical power (Lafosse et al., 2013). From these tests, the authors found the MS participants scored worse on immediate and delayed recall, which the authors attributed to the MS participants acquiring fewer words. The MS group did not score worse on recognition, but did score significantly worse on a discriminability index, meaning the MS group had more false positives in the recognition test (Lafosse et al., 2013). The meta-analytic component of list-learning and memory aspects reported in this study examined studies published in English after 1983 that compared MS to healthy controls on list-learning tests of at least 10 words. From the 50 studies analyzed, the authors found that overall MS participants performed worse on immediate, delayed, and recognition measures of memory, with immediate recall scores showing the largest effect for all MS subtypes. The MS group also showed a moderate effect size difference for the delayed free recall and recognition scores for all MS subtypes. The authors attributed the deficient immediate recall and delayed recall to an impaired acquisition processes due to poor verbal learning (Lafosse et al., 2013).

Further research is necessary to elucidate what about acquisition is impaired in MS. It is generally agreed upon that acquisition involves two main processes: encoding and consolidation. The perception and integration of sensory stimuli into a transient memory representation is assumed to occur during the encoding phase, while the strengthening of the transient memory representation into a more stable, long-term memory occurs during the early consolidation phase of memory acquisition (Ricker, 2015). Both encoding and consolidation seem to be impaired in MS (Sandry, Zuppichini, Rothberg, & DeLuca, under review). Additional investigation and parsing of these and

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related cognitive processes that underlie acquisition, as well as their neural underpinnings, will help to reveal what specifically about acquisition is impaired.

Imaging of MS-related memory impairment. Neuroimaging techniques, like magnetic resonance imaging (MRI), can be used to non-invasively assess structural brain states. MRI has shown to be sensitive in detecting MS-related brain atrophy (Preziosa et al., 2016; Rocca et al., 2015). Indeed, imaging studies in MS show substantial lesions within white matter, gray matter, in normal-appearing white matter, and in brain vasculature (Benedict, Bruce, et al., 2006; Preziosa et al., 2016; Rocca et al., 2015; Rocca et al., 2016). The correlation of MRI data with clinical assessments has shown that MS-related damage to brain structures plays a significant role in cognitive impairment (Preziosa et al., 2016). MRI research may therefore be able to aid in the understanding of MS-related memory impairment.

An early MRI study in MS found relationships between total lesion area, the size of the corpus callosum, and neuropsychological test scores (Rao et al., 1989). The study found that scores on 25 out of 34 cognitive test variables were significantly predicted by total lesion area, corpus callosum size, or both of the MRI variables together. Total lesion area served as the best predictor of performance on memory assessments, which consisted of the SRT, spatial recall test, and the story recall test, in that higher total lesion area predicted worse performance on those tests (Rao et al., 1989). Additionally, the size of the corpus callosum predicted test performance on mental processing speed and problem solving.

Subsequent studies show mixed support for a correlation between total lesion area and performance on neuropsychological tests. For instance, one study looking at T2-

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weighted images found no correlation between total lesion area and neuropsychological tests of attention, short-term memory, and working memory; although, those with secondary progressive MS showed more lesions compared to relapsing-remitting MS (Foong et al., 2000). This study attributed the lack of a correlation between lesions and neuropsychological performance to the inability of their T2-weighted images to pick up on microstructural damages in normal-appearing white matter, citing a related study (Rovaris et al., 1998) that reported a relationship between microstructural damages and cognitive impairment (Foong et al., 2000). Further studies continued to show mixed results. For example, one study found higher T1 and T2 lesion volumes in participants with memory impairment (Filippi et al., 2000) and another found that the T1 and T2 lesions load did not differ between with and without impairment (Zivadinov et al., 2001). Additional imaging research with the ability to assess microstructural MS-related neuropathology may be able to contribute to investigations of memory impairment.

Diffusion tensor imaging (DTI) is one MRI technique that is sensitive to both macro- and microstructural MS-related lesions (Rovaris et al., 2002). When tissue breaks down in the brain, as in MS-related neuropathology, barriers that are restrictive to molecular motion are degraded, minimizing directional flow or the anisotropy of molecules. An observed decrease in directional flow of molecules in the brain (decrease in anisotropy) is suggestive of lesions in the area under observation. The most commonly used measure of anisotropy is fractional anisotropy (FA), which measures the molecular flow within fiber tracts, and is on a scale of 0 (completely isotropic) to 1 (completely anisotropic) (Mori & Zhang, 2006; Pierpaoli & Basser, 1996). FA values closer to 1 are associated with less diffusion and better white matter integrity, whereas FA values closer

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to 0 are associated with more diffusion and poorer white matter integrity. One of the first studies using DTI in MS administered an extensive battery of neuropsychological assessments 48 hours after the MRI scans (Rovaris et al., 2002). Forty-seven percent of MS participants showed memory impairment (as assessed by the SRT), and mean diffusivity, a measure of average molecular motion, correlated with the symbol digit modalities test (SDMT) (Rovaris et al., 2002). The SDMT is often thought of as a test of information processing speed, but it has been shown to contain learning and memory components (Sandry et al., under review; Sonder, Burggraaff, Knol, Polman, & Uitdehaag, 2014).

Another study looked at benign MS, secondary progressive MS, and healthy controls using DTI and neuropsychological evaluations (Rovaris et al., 2008). Benign MS was defined as MS patients who have absent or low cognitive and physical disability despite a long disease duration. All results from neuropsychological testing were standardized based on percentile distribution from normal controls, with individual test scores ranging from 0 to 4, where 4 means normal performance. A score of 0 on one memory test qualified a participant as memory impaired, and scores of 0 on any three tests qualified a participant as cognitively impaired. The authors reported that 27% of benign MS participants showed memory impairment, and 19% showed total cognitive impairment (Rovaris et al., 2008). Benign MS participants also showed higher average mean diffusivity in gray matter and lower average FA in normal-appearing white matter as compared to healthy controls. Moreover, the benign MS participants without memory impairment showed lower average gray matter mean diffusivity compared to the secondary progressive MS participants. The memory impaired benign MS participants

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showed no differences in diffusion parameters when compared to the secondary progressive MS participants, suggesting they were more similar neurologically. In addition to these findings, this study supported DTI as sensitive measure for microstructural differences in MS-related neuropathology.

A more recent study used DTI analysis of microstructural white matter integrity alone to differentiate between cognitively impaired and cognitively preserved MS participants (Hulst et al., 2013). Cognitive impairment here was defined as scoring at least 2 standard deviations below the healthy control mean on at least 2 neuropsychological tests. The authors used a voxel-wise statistical analysis called tract-based spatial statistics (TBSS), which projects the FA values of all participants onto a white matter tract skeleton and then applies voxel-wise cross-subject statistics. Results showed that 49% of the investigated white matter in the cognitively preserved group had lower FA values compared to healthy controls; in the cognitively impaired group, 76% of the investigated white matter had lower FA values compared to controls (Hulst et al., 2013). Specifically, both MS groups had lower FA values in the corpus callosum, superior and inferior longitudinal fasciculus, corticospinal tracts, forceps minor, fornices, and the cingulum when compared to the healthy controls, with more severe reductions in FA values found in the MS cognitively impaired group (Hulst et al., 2013). Additionally, the cognitively impaired MS participants showed a reduction of FA values in the uncinate fasciculus, and 80% of MS participants were impaired on memory function (memory impairment was defined as scoring at least 2 standard deviations below healthy mean on 2 tests of memory). The authors note that the uncinate fasciculus connects the temporal

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lobe with the orbital and frontal cortex, suggesting that neuropathology in temporal white matter tracts may play a part in MS-related memory impairment (Hulst et al., 2013).

Another recent study corroborated these results by showing that cognitively impaired MS participants, classified as impaired if they scored below one standard deviation below the normative mean on 8 of 20 identified MACFIMS parameters, had lower FA values in the uncinate fasciculus, fornices, and the cingulum (Keser et al., 2017). The authors note that the FA values of the cognitively impaired MS in the fornix, a major output of the hippocampus, significantly correlated with the visuospatial memory, verbal memory, and the working memory components of the MACFIMS (Keser et al., 2017). More research directly examining the hippocampus in MS and its correlation to clinical assessments of memory may help us understand memory impairment in MS.

Hippocampus and MS-related memory impairment. The role of the hippocampus in memory function has been well documented (Corkin, 2013; Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957; Squire, Stark, & Clark, 2004; Wixted & Cai, 2013). Hippocampal damage related to MS neuropathology may help to explain the memory impairment observed in MS. Initial research into MS neuropathology suggested it was mainly a white matter demyelinating disease; however, advances in histological and neuroimaging techniques have revealed extensive gray matter demyelination (Geurts et al., 2007).

Immunohistochemistry, and in particular techniques that allow for the visualization of myelin, has been used to show extensive atrophy and demyelination of cortical and deep gray matter regions in MS. For example, a study using proteolipid

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protein (for lesions and demyelination) stains found that 79% of MS participants showed demyelination lesions in the hippocampus, with the majority of hippocampal lesions occurring in the molecular layer of the dentate gyrus (Geurts et al., 2007). A selection bias occurs however when you use post-mortem studies as cases examined at autopsy usually include older adults with longer disease duration totals (Geurts & Barkhof, 2008). Therefore, there is a need to assess deep gray matter lesions in-vivo to understand how they could affect the memory of living MS patients.

In-vivo cortical lesion visualization of deep gray matter structures has been difficult to obtain accurately, however, recent advances in neuroimaging techniques have begun to address this issue. For example, a study using a three dimensional double inversion-recovery (3D-DIR) technique found that 88% of MS participants showed at least one hippocampal lesion with a mean of 2.6 lesions (SD=1.8) (Roosendaal et al., 2008). Moreover, the authors reported that the 3D T2-weight images traditionally used for lesion visualization only observed 56% of the total hippocampal lesions observed by the 3D-DIR technique (Roosendaal et al., 2008). Although there are difficulties accurately assessing MS-related lesion load of deep gray matter structures, other measures of MRI can be obtained to assess the integrity of the hippocampus.

A different way imaging can assess structural integrity of gray matter structures is by looking at the volume of the structure. If atrophy occurs as a result of disease progression, then the volume for that structure will decrease. One study looking at the total volume of the hippocampus found that MS participants showed significant bilateral hippocampal atrophy compared to healthy controls, and that hippocampal atrophy correlated with poorer performance on a word-list learning task, but not with the paced

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auditory serial addition test (Sicotte et al., 2008). Another study observed a similar pattern of results, reporting MS participants with hippocampi having 7% less volume compared to controls, and a correlation between hippocampal atrophy and the CVLT-II and a correlation between hippocampal atrophy and the BVMT, measures of verbal and spatial memory, respectively (Koenig et al., 2014). A more recent study divided a group of MS participants into mnemonically impaired (51.6%) and mnemonically preserved (48.4%); participants who scored 1.5 standard deviations below the normative mean in either the SRT, a verbal memory test, or the 10/36 spatial recall test were considered mnemonically impaired (González Torre et al., 2017). The authors found that those MS classified as mnemonically impaired showed significant volume loss in the left presubiculum and subiculum, left cornu ammonis (CA) 2-3, the left fimbria, left CA4 and dentate gyrus, and the right fimbria compared to the mnemonically preserved MS and the healthy control group, who did not differ in hippocampal volume compared to the mnemonically preserved MS group (González Torre et al., 2017). The previous studies have shown that hippocampal lesions and atrophy correlate with memory impairment in MS, but more research using different techniques are necessary to assess whether there are other hippocampal abnormalities related to MS neuropathology that have so far gone undetected (Leavitt & Sumowski, 2016).

Studies using DTI, a neuroimaging technique mentioned in the previous section, could help detect microstructural abnormalities in the hippocampus related to memory impairment in MS. A recent study using DTI and traditional MRI measures compared the structure and function of hippocampi in groups of MS, healthy controls, and those with clinically isolated syndrome (CIS), which is defined as a person who has only had their

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first clinical episode suggestive of MS (Planche et al., 2016). The authors found that hippocampal volumes between the 3 groups were not significantly different but hippocampal FA was significantly different between all 3 groups. The CIS group had significantly lower FA and higher mean diffusivity than the healthy control group, while the MS group had significantly lower FA and higher mean diffusivity than both the CIS and healthy control groups (Planche et al., 2016). Mean diffusivity in the CIS group also correlated with the long-term recall section of the SRT while volume and T2-lesion load did not significantly correlate with any tests of memory. Furthermore, the study found that hippocampal mean diffusivity was able to discriminate between memory impaired and memory preserved CIS groups (classified as scoring below 1.5 standard deviations on the delayed recall section of the SRT) (Planche et al., 2016). Therefore, this study supports neurodegeneration of the hippocampus in MS-related pathology, even as early as the first clinical episode, and supports DTI as a sensitive measure of these changes.

The preceding studies in this section show how MS affects hippocampi and memory. Hippocampal pathology related to MS consists of extensive lesions, volume loss, and microstructural abnormalities. The MS-related neuropathology of the hippocampus also significantly correlates with neuropsychological tests of verbal and spatial memory. At this time, due to the narrow scope of memory that has been assessed in MS, it is unknown if other aspects of memory are affected by hippocampal degeneration caused by MS. Research that assess other aspects of memory and their correlation with hippocampal degeneration could help further our understanding of MS memory impairment.

Pattern Separation & Completion

Pattern separation is the ability to accurately discriminate between completely new stimuli and similar episodic memories, and studies suggest this ability is localized in the hippocampus (Rolls, 2016; Yassa & Stark, 2011). Pattern completion is the ability to generalize noisy or partial sensory information to a level of accurate recognition, and studies suggest this process is localized in the hippocampus (Rolls, 2013, 2016). The most current theory pertaining to pattern separation and completion is Roll's theory of hippocampal function, which outlines how the different subsections of the hippocampus compute pattern separation and completion (Rolls, 1987, 2007, 2013, 2016; Treves & Rolls, 1994). In this theory, whether or not pattern completion or separation occurs depends on the state of the CA3 subsection of the hippocampus. The CA3 subsection contains cells with recurrent collaterals, which are neurons that synapse on cells within the same system. The recurrent collateral circuitry in the CA3 cells creates an auto-associative network, in which neural patterns can become associated with themselves (Knierim & Neunuebel, 2016). This CA3 auto-association network functions based on attractor dynamics. In attractor dynamics, a stable state is created that the network system is attracted to maintain, i.e. a state that the system will revert to naturally or when not acted upon by outside input (Rolls, 2007). The activity of the CA3 network changes from its stable state based on input it receives from mossy fiber cells in the dentate gyrus and layer II cells from the perforant pathway (see Figure 1). Following is an overview of how Rolls's theory of hippocampal function was developed and then tested in both animal and human models.

Rolls's Theory of Hippocampal Function. Original research into how auto-associative networks created by recurrent collaterals could facilitate learning & memory came from a theory of neocortical functioning (Marr, 1970). The theory's main point was that the neocortex used auto-association neural networks to classify incoming information so that it can be stored with preexisting classes of information, creating and adding to abstract thoughts in the process (Marr, 1970). Based on results from the theory of the neocortex, the authors suggested that there needed to be a simpler system that did not deal with categorization or organization of information but only with simple memorizing and association of the information; this was termed the theory for the archicortex, or theory of the hippocampus (Marr, 1971). Both theories suggested that auto-association networks were formed in the neocortex and hippocampus to facilitate their respective functions (Marr, 1971). The theory of the hippocampus by Marr (1971) was then extended to include specific subparts of the hippocampus in Roll's theory of hippocampal function (Rolls, 1987). Experiments using single-cell recording methods in rhesus monkey hippocampal neurons while they were completing object-place and visual-motor response memory tasks led the authors to suggest that there is an auto-associative or attractor network created by pyramidal neurons in the CA3 layer of the hippocampus (Rolls, 1987).

Computational neuroscience is a method that utilizes biologically realistic computer models of neurons and neural networks to test and observe neuronal information processing. Using hippocampal neuroanatomy studies and computational neuroscience the theory was modified to include the presence of two distinct input networks to the CA3 attractor network: (1) mossy fiber input from the dentate gyrus, and

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(2) perforant path input directly from layer II of the entorhinal cortex (Amaral, Ishizuka, & Claiborne, 1990; Treves & Rolls, 1992). Therefore, the three main parts of the theory involves the CA3 attractor network, mossy fiber input from the dentate gyrus, and perforant path input from the entorhinal cortex, while the CA1 subsection is the main output and feedback to higher cortical areas. After identifying the possible anatomical structures in the hippocampus that mediate pattern separation and completion, it is necessary to elucidate *how* these mnemonic processes take place.

For *pattern completion*, studies suggest that any new event is given a representational firing pattern of CA3 pyramidal cells. When the information needs to be recalled, the connectivity formed by the recurrent collaterals in CA3 allows for the retrieval or recognition of a representation when only a small part of the representation is presented, which is the pattern completion process (Treves & Rolls, 1992). To induce pattern completion in the CA3 cells, studies show that cells from layer II of the entorhinal cortex that synapse directly on cells of the CA3 network are needed (Treves & Rolls, 1992).

For *pattern separation*, studies suggest that the CA3 network needs higher input from mossy fiber cells, so-named for their lack of myelin. Mossy fiber cells extend directly from the dentate gyrus and synapse on CA3 cells creating ‘detonator synapses,’ named for a single mossy fiber’s ability to depolarize the post-synaptic neuron with one or few action potentials (Treves & Rolls, 1992). The detonator synapses force a new pattern of firing within the CA3 cells that becomes associated with the new to-be-remembered event (Treves & Rolls, 1992; Treves & Rolls, 1994). Additionally, the CA3 neurons receive cortical input from the inferior temporal visual cortex (a higher level

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visual processing center), from the parietal cortex, from the superior temporal cortex (a higher level auditory processing center), and from the prefrontal cortex. This anatomical positioning of the CA3 system creates a single location where information from different cortical areas can be combined to form a “snapshot,” i.e. an episodic memory (Treves & Rolls, 1994).

A remaining question concerns what exactly needs to happen for CA3 to separate new representations or complete older ones. As mentioned previously, the CA3 cells receive input from two distinct areas, the dentate gyrus and entorhinal cortex. The difference and similarity of these inputs to CA3 cells has been suggested to determine what state the CA3 cells exhibit. The CA3 subsection does not respond to input in a linear fashion. Attractor dynamics of the CA3 region make its output somewhat resistant to changes in input, which causes CA3 cells to respond to the entire range of input in a sigmoid (S-shaped) fashion (Guzowski, Knierim, & Moser, 2004). Therefore, when the change in input to CA3 is in a small range, the network activity reflects pattern completion. For example, when weak input from entorhinal cortex reaches the CA3 network via the perforant path, the CA3 network is pushed into completing the pattern of an old memory. However, when the change in input to CA3 is sufficient enough to reach an inflection point, as when mossy fiber detonator synapses fire upon CA3 cells, pattern separation ensues (Knierim & Neunuebel, 2016). For example, when an animal is placed in a very familiar environment, the change in input to the CA3 network is in the smaller range, and thus, its activity stays in the pattern completion range. However, when an animal enters a sufficiently novel environment, and the change in input to the CA3 network is sufficiently large to reach the inflection point between completion and

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separation, the activity in the network exhibits pattern separation (Knierim & Neunuebel, 2016). Therefore, it is not the dentate gyrus or perforant pathway collaterals per se that are responsible for pattern separation or completion, but instead it is how their difference in input to the CA3 network influences whether or not the CA3 separates or completes patterns (Knierim & Neunuebel, 2016; Rolls, 2016).

Together, these three components are theorized to be responsible for mediating pattern separation and completion in the brain. The introduction of this theory has propelled many areas of research into the topic. The following sections will describe research aimed at testing Roll's theory of hippocampal function.

Animal Models of Pattern Separation & Completion. Studies with animals using various methodologies have mostly supported the functions of the hippocampal subsections in pattern separation and completion laid out by Roll's theory. One of the first studies used infusions of diethyldithiocarbamate (DDC) to selectively and reversibly dysregulate hippocampal mossy fiber synapses in one group of mice, another group of mice received a control infusion drug, Ca-ethylenediamine tetraacetic acid (Ca-EDTA), and one group of control mice received no infusions while they all completed trials of the Morris Navigation Task over multiple weeks (Lassalle, Bataille, & Halley, 2000). The Morris Navigation Task involves placing a rat or mouse in a pool of water in which an invisible platform exists that the animal must find in order to avoid treading water for air. The animal can use various cues in the pool environment to learn where the platform is located over trials and improve upon escape latencies. The deregulatory effects of DDC on mossy fibers lasted 30- to -45 minutes, which meant they were reversible. Therefore, the authors rotated the infusion treatments between the DDC and Ca-EDTA groups after

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one week to test for reversal effects. The authors found that the DDC mice were unable to learn the location of the platform in the maze during their first week of training, whereas the control and Ca-EDTA groups were able to learn the location of the platform. When the infusion groups were rotated during week two, the previously DDC mice, whom now receive Ca-EDTA infusions, were able to learn the location of the platform and even improve upon their escape latencies (Lassalle et al., 2000). Moreover, mice that received Ca-EDTA during the first week and then switched to DDC infusions for the second week did not forget where the platforms were and displayed normal performances, suggesting that mossy fiber input is not necessary for recall (Lassalle et al., 2000). To test an alternative hypothesis that selective inactivation of mossy fibers with DDC disrupts memory consolidation instead of memory formation, a second experiment was performed where post-trial DDC injections were used to block activity of mossy fibers for 45 minutes after the acquisition of spatial information. The second experiment found no differences in learning and recall between mice that received either DDC or Ca-EDTA immediately after each learning session (Lassalle et al., 2000). The authors conclude that both experiments support the mossy fiber synapses' role in the learning process while disassociating the mossy fibers from consolidation and recall processes, supporting Rolls's model. However, this is not without limitations. The study did not perform any checks to assess whether the DDC dysregulation truly affected the activity of the mice mossy fiber cells. More research is necessary, therefore, to provide evidence to support Rolls's theory.

Another study used electrolytic lesions of the perforant pathway input and neurotoxic lesions of the dentate gyrus input to dissociate the roles of the inputs to the

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CA3 network with respect to encoding and retrieval processes in rats placed in a Hebb-Williams Maze modified to facilitate spatial learning (Lee & Kesner, 2004). A Hebb-Williams Maze consists of a square area with dynamic internal walls. One corner is designated the start of the maze and the other contains a dietary reward; the animal must navigate the maze in order to receive this reward. The results from this study showed that generally, both lesion groups had similar learning deficits in terms of total errors compared to the control group, however, the source of the deficits was observed to be from different mnemonic processes (Lee & Kesner, 2004). Specifically, the group with dentate gyrus lesions was not efficient at reducing the number of errors within the first day, compared to the perforant path lesioned group. On the other hand, the perforant path lesioned group was not efficient at carrying over their first day performance to the next day, exhibiting more errors on the second day than the previous (Lee & Kesner, 2004). The authors note that the modified Hebb-Williams maze used in the study appears to require the rats to separate two paths based on memory; one path leads to the goal and another lead to a dead-end, with the correct path having a narrower entrance than the wider, incorrect path. In their discussion, they suggest pattern separation might be essential in the selection process between these paths and could explain why the dentate gyrus lesioned group was unable to reduce their number of errors within a day efficiently, while the perforant path lesioned group did not show this inability to reduce errors on the first day (Lee & Kesner, 2004). However, the perforant path lesioned group did show more errors the following day, suggesting its involvement in retrieving memories after a longer period of time (Lee & Kesner, 2004).

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Another more recent study used a strain of mice called “knockout mice” (because a gene is selectively “knocked out”) that selectively lacked the gene coding for an essential subunit of a NMDA receptor NR1 located in dentate gyrus cells (McHugh et al., 2007). The study used a contextual fear-conditioning experimental paradigm, which involves placing mice in a room that can be associated with a footshock based on the context (e.g. type of floor, smell, color, lighting, etc.). With this paradigm, animals placed in rooms with contexts that have been associated with footshocks freeze in anticipation of the associated shock. The study showed that there was no difference between control and knockout mice in learning a footshock pairing within a single chamber, termed chamber A. But when the authors introduced a similar yet novel chamber B (identical metal grid floor to chamber A but with unique odors, roof, and lighting) the knockout mice had trouble discriminating between the two different chambers, freezing at similar rates in both chamber A and B, while the control group learned to discriminate between the chambers, showing less freezing in chamber B over time, supporting the theorized role of the dentate gyrus in accurately discriminating between similar stimuli (McHugh et al., 2007).

A very recent review looked at studies conducted over the past decade that used double-rotation experiments and neurophysiological recordings of hippocampal afferents (entorhinal cortex and anterior thalamus), intrahippocampal regions (dentate gyrus and CA3), and the hippocampal output layer (CA1) to evaluate Roll’s theory in rats (Knierim & Neunuebel, 2016). The double-rotation experiments involved a track divided into 4 quadrants, each with a different color and texture. The track contained local salient cues on the track and global salient cues on a surrounding curtain. The term “double rotation”

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refers to the manipulation of the local reference cues so that they are placed in varying degrees of conflict with the global cues over multiple trials (Knierim & Neunuebel, 2016). The studies observed neuronal activity using various direct electrophysiological recording techniques in the aforementioned areas while these cues were matched and mismatched on different trials to create similar yet different environments. The review found that with small manipulations to the environment, CA3 network activity changed less than the CA1 output representation, which was taken as evidence for pattern completion. Conversely, when there was a large change to the environment, the CA3 network activity changed more than the CA1 output, suggesting pattern separation (Knierim & Neunuebel, 2016). Moreover, these studies observed increased activity in the dentate gyrus during pattern separation activity in the CA3 network coinciding with more novel looking environments (Knierim & Neunuebel, 2016). The review concludes by stating that the studies support the model's predictions that the dentate gyrus initiates pattern separation in the CA3 network and that the CA3 network behaves as an attractor network with pattern completion abilities as well. However, these conclusions do not come without caveats, which are as follows: the functional role of the CA2 region has so far been ignored and needs to be studied; other inputs into the hippocampus need to be evaluated, such as inputs from the septum, perirhinal cortex, and brainstem; and the role of adult neurogenesis in the dentate gyrus needs to be assessed and incorporated into the existing theory framework (Knierim & Neunuebel, 2016). Lastly, the review emphasizes the need to translate and test results from animal studies to human subjects within the framework of Rolls's theory of Hippocampal function using neuroimaging techniques (Knierim & Neunuebel, 2016), although there has been some research in this area.

Assessing Pattern Separation & Completion in Humans. Translating results from animal research necessitates the creation of a task that assesses pattern separation and completion behavior in humans. The existing literature on pattern separation and completion in humans is sparse as there are not many behavioral paradigms available. Since the first study in humans, only two tests have emerged with consistent use, the spatial pair distance task (Stark, Yassa, & Stark, 2010) and the Mnemonic Similarities Task (MST) (Stark, Yassa, Lacy, & Stark, 2013). The spatial pair distance task has been used less frequently in the literature; therefore, this section will focus on the MST for assessing pattern separation and completion in humans.

The first study designed to assess pattern separation and completion behavior in humans was also the first study to use what would eventually become the MST (Kirwan & Stark, 2007). In its initial use, the task was presented to participants in a continuous recognition paradigm in which everyday objects were presented in color one at a time for 2500 ms with an inter-trial interval of 500 ms. Each block over time presented images that were slightly different but similar to previously presented pictures (similar), exact repeats of previously presented pictures (old), and completely new pictures (new); participants then had the forced choice option of “old,” “similar,” and “new” at the end of each trial. The authors reasoned that the behavioral consequence of pattern separation is manifested in the ability to mnemonically distinguish between two stimuli presented in this paradigm (Kirwan & Stark, 2007). Therefore, if a participant correctly chooses “similar” in response to an object that is slightly different but similar to a previously presented object (correct lure rejection), then pattern separation correctly occurred. On the other hand, if a participant answers “old” to a similar object (lure false alarm), then it

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would indicate a failure of pattern separation and the incorrect occurrence of pattern completion. Alternatively, if participants correctly responded “old” to repeated images (hits) this suggested a correct occurrence of pattern completion (Kirwan & Stark, 2007). Using high-resolution neuroimaging techniques, the authors found that hippocampal activity was correlated with behavioral performance on this task and was able to distinguish between response types (Kirwan & Stark, 2007). The investigators observed that activity in the dentate gyrus, CA3, and CA1 subsections of the hippocampus was significantly lower for lure false alarms when compared to both hits and correct lure rejections (Kirwan & Stark, 2007). The authors suggest that this pattern of activity is consistent with a “recall to reject” interpretation (Kirwan & Stark, 2007).

The “recall to reject” interpretation suggests that in order for participants to correctly reject similar lures, an accurate representation of the original stimulus must be formed in memory, and necessarily, an accurate retrieval of the representation must be performed for comparison. In this reasoning, when the participant is presented with a lure, they must perform pattern completion of the older image to compare with the currently presented stimulus to make a decision. Therefore, errors could occur in the encoding or retrieval phase of the original stimulus as pattern separation here depends on accurate pattern completion. Furthermore, the “recall to reject” interpretation suggests it is impossible in this paradigm to differentiate between pattern completion and separation processes (Kirwan & Stark, 2007).

A study that followed used a modified version of the previous task to assess pattern separation and completion in humans undergoing high-resolution fMRI scans (Bakker, Kirwan, Miller, & Stark, 2008). In the modified version, participants performed

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an incidental encoding task, which involved them looking at pictures of everyday objects and deciding whether or not they were indoor or outdoor items. In doing so, the authors argued that the “recall to reject” strategy may be mitigated because the task was not overtly mnemonic and the participants would also be unaware of the pattern separation demands of the task, as there were no questions about whether objects were old, similar, or new (Bakker et al., 2008). To visualize pattern separation or completion, the study used high-resolution fMRI techniques. Despite the high-resolution imaging techniques, this study was still unable to isolate the CA3 subregion from the dentate gyrus, instead grouping them together. Therefore, the authors’ reasoning behind being able to differentiate pattern separation and pattern completion neuronal activity is as follows: if a region is engaged in pattern separation, then that region will show activity resembling activity for the first presentation of an object; alternatively, if a region is engaged in pattern completion, then that region will show activity consistent with activity that occurs when a participant sees the repetition of an object (Bakker et al., 2008). The study found that activity in the CA3/dentate gyrus sections was significantly different when presented with a lure as compared to a repeat presentation. Additionally, the authors found no significant difference in CA3/dentate gyrus activity between lure presentations and first presentations of objects (Bakker et al., 2008). The observations provide evidence for pattern completion and separation occurring in the CA3/dentate gyrus under different patterns of activity. However, the inability to isolate the two subregions is limiting and does not allow for further discrimination concerning which subregion, CA3 or the dentate gyrus, is associated with pattern separation or completion in humans.

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A follow up study by the same lab intended to replicate and extend the previous research by modifying the task further to include parametric variations in mnemonic similarity (Lacy, Yassa, Stark, Muftuler, & Stark, 2011). The incidental encoding task that was used in the previously mentioned study (Bakker et al., 2008) had the lures evaluated by a separate study for their mnemonic similarity creating two groups of high-similarity lures and low-similarity lures (Lacy et al., 2011). The authors looked at these two groups along with first presentations, and repetitions of items in relation to activity in CA3/dentate gyrus and CA1 areas. The authors predicted that small changes in similarity (high-similarity) would elicit activity in the CA3/dentate gyrus similar to that of first presentations, whereas CA1 activity would vary incrementally with changes in similarity. Additionally, the authors predict that activity in CA3/dentate gyrus and CA1 areas should converge when changes in similarity are large enough (low-similarity), which would be suggestive of pattern separation occurring in both regions (Lacy et al., 2011).

Results showed that completion-like activity (activity during lures that was different from first presentations but not from repetitions) occurred in regions of the CA1, and that separation-like activity (activity during lures that was different from activity during repetitions but not from first presentations) occurred in regions of the CA3/dentate gyrus, corroborating past research (Lacy et al., 2011). When looking at how changes in input (mnemonic similarity) affected regional activity responses, results showed a significant difference in how CA1 and CA3/dentate gyrus responded. The CA1 region responded in a graded fashion with no significant response to repetitions, small response for high-similarity lures, moderate response to low-similarity lures, and a large response to the first presentation of items (Lacy et al., 2011). On the other hand, CA3/dentate

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gyrus activity response to high-similarity lures was significantly higher than CA1 regions, while showing no significant differences in activity between the two regions for low-similarity lures or first presentations (Lacy et al., 2011). The authors conclude that these results replicate previous work by Bakker et al. (2008) and extend findings by assessing regional differences in activity in response to the level of mnemonic similarity in lures (Lacy et al., 2011). The observed results support the authors' predictions by showing a difference between CA1 and CA3/dentate gyrus activity for small changes in input (high similarity), but no difference in activity for larger changes (low-similarity or first presentations) (Lacy et al., 2011). These results suggest that the CA3/dentate gyrus region is specifically attuned to pattern separation based on its pattern of activity, and that both the CA1 and CA3/dentate gyrus sections show activity representing pattern separation processes when differences in input are large enough or an object is being presented for the first time.

Despite its findings, the study suffers from similar limitations to the one it was designed to replicate. Specifically, this study has no overt tests of memory and the task still does not completely control for the “recall to reject” strategy. Additionally, high-resolution imaging techniques used in both studies are still unable to differentiate between the CA3 and dentate gyrus subregions, which is necessary to accurately assess the roles each subsection plays in separation and completion. Future studies should look to modify the task further so that it is a true mnemonic test of pattern separation and completion while also mitigating the occurrence of a “recall to reject” strategy, and to use advancements in imaging techniques to eventually isolate the CA3 and dentate gyrus.

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The Mnemonic Similarities Task. The most current version of the MST (still called the behavioral pattern separation task at time of publication) was presented in a study that looked at memory impairment associated with healthy aging and mild cognitive impairment (Stark et al., 2013). In an additional experiment, the authors tested out a newer version of the MST in a young population to create a “similarity metric” for each stimulus-pair showing the degree of change in input between old items and lures (Yassa, Lacy, et al., 2011). This was done in order to assess the degree of change in input needed to induce pattern separation in the older population experience age-related pattern separation deficits (see next section) (Yassa, Lacy, et al., 2011). This additional experiment led to the current state of the MST that is widely used today.

The MST consists of two phases: an encoding phase and a surprise recognition memory test (Figure 2). During the encoding phase, participants see pictures of everyday objects and are asked to judge whether the items are indoor or outdoor items. Immediately after the encoding phase, the participants receive instructions for a surprise recognition memory test saying they must identify each item they see as either “Old,” “Similar,” or “New.” For the presentation of objects in the recognition memory test, a third of the images are repetitions (targets), a third are completely new objects (foils), and another third are similar to images seen in the encoding phase but are slightly different (lures) (Stark et al., 2013). Pattern separation performance is calculated using the ratio of “Similar” responses to lure items (correct responses) minus the “Similar” responses given to the foils (to adjust for possible bias a participant may have to respond “Similar”). This calculation was termed the behavioral pattern separation score but was later changed to

the “lure discrimination index” as the exact validity of the test and the terminology used has been debated.

An inconsistency in the literature exists concerning the name of the task and its scores, and it is only ever explicitly expressed in a footnote in a paper looking at the effect of aging on pattern separation (Stark, Stevenson, Wu, Rutledge, & Stark, 2015). Originally, the above task was termed the behavioral pattern separation task, but critiques about what the test is actually assessing led researchers to discuss and admit that other processes may be involved in executing the task (Stark et al., 2015). The authors henceforth refer to the task as the MST because they can only definitively say that the task assess the ability to discriminate between mnemonic similarities. In other words, the task reflects the ability of participants to discriminate lures using recognition memory. Therefore, the present paper will hereafter refer to it as the MST, and the pattern separation scores from the task as lure discrimination scores (LDI). Despite this, the authors continue to contend that the ability to discriminate between mnemonic similarities reflects pattern separation processes (Stark et al., 2015).

Assessing Pattern Separation & Completion in Aging Samples. Studies on rodents, primates, and humans have all shown that the dentate gyrus is one of the primary areas affected by normal aging (Barnes, 1979; Gazzaley, Siegel, Kordower, Mufson, & Morrison, 1996; Small, Tsai, DeLaPaz, Mayeux, & Stern, 2002; Yassa & Stark, 2011). One of the first studies looking specifically at pattern separation deficits in a healthy aging human sample found pattern separation deficits that resulted in a bias for the older individuals to pattern complete incorrectly (Yassa, Lacy, et al., 2011). This study also found that the bias to incorrectly pattern complete in older adults was correlated with

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hyperactivity in the CA3 region (Yassa, Lacy, et al., 2011). The shift in bias from pattern separation to pattern completion is suggested to be a specific age-related impairment and has been supported by rat models of neurocognitive aging (Wilson, Gallagher, Eichenbaum, & Tanila, 2006). The bias to pattern complete corroborates animal models of ageing that found a correlation between aging rats unable to complete the Morris water maze task and hyperactivity of the CA3 network (Wilson et al., 2006; Wilson et al., 2003). Researchers suggested that the over-excitation of the CA3 network was due to a deteriorated perforant path input, and therefore faulty dentate gyrus input (Barnes, Rao, & Houston, 2000). Accordingly, research looking at perforant path integrity in normal aging humans could help to elucidate what happens to cause the pattern completion bias in healthily aged individuals.

A study using ultra-high-resolution diffusion tensor imaging looked at perforant path integrity in healthy aging humans (Yassa, Muftuler, & Stark, 2010). The study quantified perforant path integrity by looking at the direction, magnitude, and anisotropy of tensors using FA values (Yassa et al., 2010). Results showed that the perforant pathway integrity was worse in a sample of older adults (mean age of 70 years) compared to young adults (mean age of 21 years) (Yassa et al., 2010). Additionally, there was no difference between the groups in the entorhinal cortex and an *a priori* control area, the alveus, suggesting the degradation of the perforant pathway related to aging is not part of a global phenomenon (Yassa et al., 2010).

A follow-up study by the same lab found that the perforant path degradation related to aging correlated with hyperactivity in the CA3/dentate gyrus network (still unable to be isolated) and correlated with lure discrimination deficits (Yassa, Mattfeld,

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Stark, & Stark, 2011). The authors found no differences when stimuli in the task were very different (low-similarity). However, when the stimuli were highly similar, the activity in the CA3/dentate gyrus of older adults was lower in comparison to the young adults (Yassa, Mattfeld, et al., 2011). The requirement for stimuli to have increased dissimilarity in order for proper pattern separation to occur, i.e. a bias for pattern completion, is referred to as “representational rigidity” because of the greater resistance to a change in input (Yassa, Mattfeld, et al., 2011). Using diffusion tensor imaging, the authors found that rigidity, or hyperactivity in CA3/dentate gyrus, significantly correlated with FA values in the CA3/dentate gyrus area, suggesting that microstructural changes in this region contribute to the impairment, and no correlations were found to other areas (Yassa, Mattfeld, et al., 2011). There was also a direct correlation between FA values of the perforant pathway and lure discrimination: lower the FA values were associated with worse performance on the MST (Yassa, Mattfeld, et al., 2011). Lastly, the authors looked at the functional coupling of the CA3/dentate gyrus with the entorhinal cortex and found a significant correlation between the functional coupling and rigidity in the CA3 region, suggesting that rigidity in the CA3/dentate gyrus is related to degraded signals between the entorhinal cortex and the hippocampus (Yassa, Mattfeld, et al., 2011). These results support the age-related bias to pattern complete that is correlated with CA3 hyperactivity. Additionally, the results suggest that microstructural abnormalities in both the CA3/dentate gyrus and perforant pathway contribute to the pattern completion bias, or representational rigidity seen in age-related memory decline (Yassa, Mattfeld, et al., 2011). Together these results suggest a general circuitry issue between the entorhinal cortex and hippocampal subregions that occurs in the healthy aging population.

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In one study, the authors wanted to know if pattern separation could be used as a sensitive marker of memory changes related to aging (Stark et al., 2013). The study used the MST to assess pattern separation via LDI scores of 5 groups: participants aged 20-39, participants aged 40-59, participants aged 60-75, participants aged 75-89, and older participants (mean age=74.4) with a diagnosis of amnesic mild cognitive impairment (Stark et al., 2013). The task being used can also be a measure of recognition memory performance, so the authors looked at this first by operationalizing correct recognition as the percent of “Old” responses to repetitions minus the percent of “Old” responses to completely new objects (hits minus false alarms). The authors found no differences between all healthy age groups in recognition scores derived from the task (Stark et al., 2013). The authors found a significant effect of age with a linear trend, and a negative correlation between age and LDI scores (Stark et al., 2013). In support of recent evidence on aging, the authors found that older participants were more likely to respond “Old” to lure items, i.e. older participants showed a bias towards pattern completion (Stark et al., 2013). Based on these results so far, recognition performance could not differentiate between age groups.

Going further, the authors split the healthy 60+ aged participants into thirds using Rey’s Auditory Verbal Learning Test delayed word list recall scores. The top third, who recalled 12-15 out of the total 15 words on the delayed recall, were termed the “Age-Unimpaired” group, while the bottom third, those who recalled only 5-8 words on this delayed recall test, were classified as the “Aged-Impaired” group (Stark et al., 2013). The authors compared these two groups, the top and bottom third of performers, to the group with amnesic mild cognitive impairment on both recognition and pattern separation

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scores. They found no evidence for a recognition score difference between the Impaired and Unimpaired groups, however the amnesic group was significantly worse than both the Impaired and Unimpaired aged groups on recognition scores (Stark et al., 2013). In contrast, when the authors looked at LDI they found that the Impaired group performed significantly worse than the Unimpaired group, yet found no significant differences in LDI between the Impaired group and the amnesic group (Stark et al., 2013). Finally, looking at the level of mnemonic similarity of the stimuli, which was assessed in a previous study (Yassa, Lacy, et al., 2011), the authors found that as age increased, greater changes in input were necessary to induce pattern separation processes (Stark et al., 2013).

The results from this study corroborate previous findings (Stark et al., 2013) that suggest a bias to pattern complete occurs in the healthy aging population. This bias is also known as “representational rigidity” in references to the rigidity of the CA3 network. Due to this bias, as participants age, a greater dissimilarity is needed between similar stimuli in order for correct lure discrimination to occur, which is supported here. In the results, only the LDI, an indicator of pattern separation abilities, was able to distinguish between age groups and the group with amnesic mild cognitive impairment. Therefore, this study also showed how tasks designed to assess pattern separation may be better at assessing memory impairment than traditional measures of memory impairment, like recognition memory (Stark et al., 2013). Notably absent from this study, however, is neuroimaging assessment of how these findings relate to neuronal activity in the hippocampal subfields and the surrounding cortical areas.

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A recent study used ultra-high in-plane resolution DTI to assess the relationship between diffusion parameters of the perforant pathway and broader medial temporal lobe network with pattern separation in a healthy aging population (Bennett & Stark, 2016). A main goal of this study was to assess the specificity of the relationship between the perforant path and mnemonic discrimination with respect to other cognitive domains and white matter tracts (Bennett & Stark, 2016). To do this, the authors assessed participants on pattern separation using the MST and on other cognitive domains using ten neuropsychological tests. The authors then used principle components factor analysis to identify five factors capturing different cognitive constructs: two factors captured recall memory; one independent factor captured mnemonic discrimination (using various indices taken from the MST only); and two other factors captured executive functioning and working memory (Bennett & Stark, 2016).

The authors first found significant age-related declines in perforant path diffusion and anisotropy, and fornix anisotropy; after calculating for global diffusion and anisotropy metrics, a multiple regression analysis showed that this age-related decline in perforant path and fornix diffusion and anisotropy measures was still significant after controlling for global measures (Bennett & Stark, 2016). The authors found that perforant path diffusion significantly predicted the mnemonic discrimination factor, while not significantly predicting any other factor (Bennett & Stark, 2016). Stepping back to the broader medial temporal lobe network, the authors decided to look at the hippocampal cingulum, the fornix, and a control tract, the corpus callosum, for further examination. Using separate multiple regression models to assess whether tract integrity in these places predicted any of the five factors, while controlling for global integrity, the authors found

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that diffusion in the hippocampal cingulum significantly predicted the mnemonic discrimination factor (Bennett & Stark, 2016). Additionally, hippocampal cingulum anisotropy was also barely a significant predictor of a factor consisting primarily of verbal recall memory measures (Bennett & Stark, 2016). Fornix measures did not significantly predict any factors but its anisotropy measure did significantly correlate with the lure discrimination index. The control corpus callosum tract did not significantly predict any factor (Bennett & Stark, 2016).

Notably, according to Rolls's theory, information from the hippocampus needs to be retrieved to affect other cortical areas for the formation of a complete neocortical memory representation (Rolls, 2016). Rolls therefore describes a "theory of recall by backprojections," which suggest the existence of a backprojection from the hippocampus to the neocortex via the broader temporal network mentioned in the previous study by Bennett and Stark (2016). Therefore, there is evidence that although it plays a large part, the hippocampus alone is not enough for pattern separation ability, and that research should also account for the broader temporal network.

These results support past research that consistently identify an age-related decline in pattern separation abilities. Results corroborated past research that perforant pathway integrity plays a role in the pattern separation deficit seen in the healthy aging population. Furthermore, the broader medial temporal lobe network, including the hippocampal cingulum and fornix, may also play more important roles in pattern separation than previously thought given these results. More research into how the broader medial temporal lobe network functions with hippocampal subregions is needed to better understand the neural underpinnings of behavioral pattern separation. Along

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with other aspects of memory, pattern separation deficits are observed in the healthy aging population. The age-related pattern separation deficit is characterized by a shift in bias to pattern completion, which has also been referred to as “representational rigidity.” The term rigidity refers to the greater change in input needed to elicit pattern separation-related activity in the CA3 network. The rigidity in the CA3 network has been correlated with the bias to pattern complete and to the integrity of para-hippocampal and broader medial temporal lobe white matter tracts, like the perforant path, hippocampal cingulum, and fornix. More research into whether pattern separation deficits occur in other populations, and the characterizations of any deficiencies could help to elucidate how the pattern separation process emerges from the hippocampal subregions and the broader medial temporal lobe network.

Current Research

It is widely supported that, along with physical disability, the disruption of neural transmission in people with MS can cause profound and wide-ranging cognitive impairments (Chiaravalloti & DeLuca, 2008; Rao, Leo, Bernardin, et al., 1991). Long-term memory is one commonly impaired cognitive function (Chiaravalloti & DeLuca, 2008; Rao et al., 1993; Rao, Leo, Bernardin, et al., 1991; Rocca et al., 2015) and localized to the memory acquisition process (Lafosse et al., 2013). Hippocampal changes are also well documented and related to memory impairment (Hulst et al., 2015; Kiy et al., 2011; Planche et al., 2016; Sacco et al., 2015; Sicotte et al., 2008; Sumowski et al., 2017); however, existing neurocognitive measures are somewhat non-specific (Sandry, Akbar, et al., 2016). Research assessing other aspects of memory acquisition, for example, pattern separation, and the correlation with neurological changes may lead to

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earlier and more specific measurement of memory impairment in MS. The current study will assess pattern separation in MS using the MST along with traditional neuropsychological assessments of memory. Ultimately, the study aims to investigate the sensitivity of these measures in identifying volumetric hippocampal changes and microstructural differences in hippocampal inputs.

Gray Matter Hypotheses

Hypothesis 1.A. A positive relationship between hippocampal volumes and pattern separation scores whereby smaller volumes will be correlated with lower LDI scores.

Hypothesis 1.B. A positive correlation between hippocampal volumes and traditional neuropsychological measures of memory (verbal and visual), whereby smaller volumes will be correlated with lower memory scores.

Hypothesis 1.C. Given pattern separation is localized to the hippocampus, LDI scores should account for more variance in hippocampal volumes compared to traditional neuropsychological measures of memory.

White Matter Hypotheses

Hypothesis 1. MS participants with differing pattern separation scores (low compared to high LDI scores; median split) will show different diffusion parameters indicative of white matter differences.

Hypothesis 2. MS participants who score low on the pattern separation measure will show greater diffusion (lower FA values) in medial temporal regions than MS participants with higher pattern separation scores.

Hypothesis 3. White matter tracts connecting to the hippocampus, e.g. the cingulum and the temporal part of the superior longitudinal fasciculus, will show lower fractional anisotropy values in the MS participants with lower pattern separation scores.

Methods

Participants

A sample of 16 MS participants were recruited through Kessler Foundation Research Center located in East Hanover, New Jersey as part of a larger two-day study of neurocognitive functioning in MS. Participants received monetary compensation (\$125.00) for their time. Participants did not have an exacerbation and were not taking a corticosteroid within the past month and reported no learning disabilities, history of serious psychiatric illness, other neurologic conditions, or a history of drug abuse. All participants were fluent in English. Participants underwent two days of neuropsychological and computer-based testing as a part of a larger study of memory impairment. Two participants were excluded from all analyses due to a low response rate on the MST, bringing the total participants to 14.

Behavioral & Neuropsychological Tests

Mnemonic Similarities Task. The MST, formerly known as the behavioral pattern separation task, was downloaded from the Stark Lab Website (<http://faculty.sites.uci.edu/starklab/>) and run on a Lenovo Edge running a 64-bit Windows 10 operating system with 1920 x 1080 resolution, 60p Hz refresh rate, and an Intel® Core™ i5-6200U CPU at 2.30GHz, 2.40 GHz.

The first phase of the task (Fig. 1A) consists of 128 color photographs of everyday day objects on a completely white background. In this phase, participants view

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the items for 2 seconds (with a 0.5 s interval) and make a response about whether the item is an indoor item or an outdoor item via keyboard keys. Immediately following this encoding task, the participants watch a video giving them instructions on the surprise recognition phase. In this phase participants see another 192 color items on white background except this time 64 items are exact repeats from the first phase (correct response: “old”), 64 items are lure items that look similar to old items but are slightly different (correct response: “similar”), and 64 completely novel foils never seen before in the experiment (correct response: “new”). Participants respond to each item they see with either “old”, “similar”, or “new” using keyboard keys. Pattern separation performance is assessed via the LDI score which is computed by subtracting the ratio of “similar” responses given to new items from the ratio of “similar” responses given to similar items, to correct for response bias. MST recognition scores are computed by subtracting the ratio of “old” responses to foils from the rate of “old” responses to targets (Stark et al., 2015; Stark et al., 2013).

Hopkins Verbal Learning Test – Revised. The Hopkins Verbal Learning test (HVLT) is a brief verbal test of memory containing a delayed recall trial in the revised version, which is used here (Benedict, Schretlen, Groninger, & Brandt, 1998). In the administration, 12 words are read out loud at a 2-second interval. Immediately after, the participant is instructed to recall as many words as possible in any order while the administrator records the responses. The learning trial is repeated twice more, with all 12 words being read aloud and the participant free recalling as many words in any order each time. Then, the participant is instructed not to forget the words, as they may be tested on them again later, and a 20-25-minute interval involving other non-verbal tests

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follows. After the delay, participants are asked if they remember the lists they tried to learn previously and are then prompted to recall as many words in any order. Following the delay recall, a list of 24 words are read aloud, included are the 12 words from the list-to-be-learned (targets) and 12 new words (6 semantically related to the targets and 6 not semantically unrelated to the targets). The participant responds with either “yes” or “no” as to whether the words were from the original list or not while the administrator records. It is important to note that the list of 12 words to be learned can be semantically divided into three categories (e.g. food, clothes, tools, etc.). Once completed, the HVLT scores include three learning trials with immediate recall scores, one delayed recall trial score, and a recognition discrimination index (RDI) computed from the recognition trials (number of true-positives minus number of false-positives) (Benedict et al., 1998).

Brief Visuospatial Memory Test – Revised. The Brief Visuospatial Memory Test revised edition (BVMT-R) assesses visuospatial memory over a short period of time (Benedict, Schretlen, Groninger, Dobraski, & Shpritz, 1996). Administration involves the 10 second presentation of six geometric abstract visual designs on 8 x 11-inch paper in a 2 x 3 columns-to-rows matrix. Immediately after presentation, the participant is given a blank sheet of paper with a pencil and instructed to draw as many shapes they can recall in the correct place on the page with no time limit. The first learning trial is repeated twice more exactly the same way. After the 3 learning trials, the participant is told not to forget the shapes, as they may be asked about them later, and a 20-25-minute interval consisting of non-visuospatial tasks ensues. After the delay, the participant is given another blank page and asked to draw as many shapes as they can remember. Then, as a recognition test, 12 shapes, 6 from the original list and 6 completely new shapes, are

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shown from a recognition flip-book and the participant is instructed to say “yes” if the shape is from the original list or “no” if it is not. Lastly, the participant completes a copy trial in which they have a chance to draw the shapes with the original stimulus matrix in view.

The BVMT-R yields scores for each immediate recall trial, the delayed recall trial, and the recognition trial. Each trial, except for recognition, has a total of 12 points that can be attained, 2 points per shape. The shapes are scored for accuracy of drawing (1 point) and accuracy of location on the page (1 point). For recognition, each false-positive (6 points) is subtracted from each true-positive (6 points) to yield a recognition score (high score of 6) (Benedict, 1997).

Digit Span Test. The Digit Span Test (DST) is part of a larger battery of tests from the Wechsler Adult Intelligence Scale, fourth edition (Wechsler, 2008). The DST involves three parts: forward, backward, and sequencing. In DST forward section, numbers are read out loud and the participant is instructed to recall the numbers in the same order they heard them. As the administration proceeds, the length of the numbers grows and the administration ends once the participant does not properly recall two consecutive trials (one item). For the DST backwards section, the same rules apply except the participant recalls the numbers backwards. For DST sequencing, the participant must recall the numbers in ascending order, from lowest to highest. For each correct trial, the participant earns a point with a total of 16 possible points for each section (totaling 48 possible points) (Wechsler, 2008).

The Symbol Digit Modalities Test. The oral Symbol Digit Modalities Test (SDMT) (A. Smith, 1982) is a brief test that has been associated with working memory,

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processing speed, and learning & memory (Benedict, Cookfair, et al., 2006; Rao, Leo, Ellington, et al., 1991; Sandry et al., under review; A. Smith, 1982). Administration of the test involves the presentation of a series of nine symbols that are each paired with a single digit in a key at the top of an 8.5 x 11-inch paper. The remainder of the paper contains the symbols from the key in boxes with empty boxes underneath. During a 90-second time limit, participants call out digits associated with each symbol as fast as they can while the administrator records their responses. The SDMT yields one main score: the number of correct responses in 90 seconds (A. Smith, 1982).

Wechsler Test of Adult Reading. The Wechsler Test of Adult Reading (WTAR) is a brief assessment of premorbid verbal intelligence (Holdnack, 2001; Venegas & Clark, 2011). Administration involves the presentation of a sheet of 50 numbered words, with 25 words in two columns. The participant pronounces each word, in order, even if he or she is unfamiliar with the word while the administrator scores each response. Each word is worth one point, giving the test a total of 50 attainable points.

Multiple Sclerosis Functional Composite. The Multiple Sclerosis Functional Composite (MSFC), was developed by the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force for the purpose of measuring cognitive and physical impairment in MS continuously. Traditionally the composite involves three parts: the Timed 25 Foot Walk (T25FW), the 9-Hole Peg test (9HPT), and the Paced Auditory Serial Addition Test (PASAT) (Cutter et al., 1999; Fischer, Rudick, Cutter, & Reingold, 1999). However, studies questioning the validity of the PASAT as a cognitive measure have used the SDMT in its place (Brochet et al., 2008; Drake et al., 2010; Sandry, Paxton, & Sumowski, 2016; Sonder et al., 2014). The current study uses the SDMT in

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place of the PASAT, with SDMT normative values and MSFC equation from Drake et al. (2010) to compute the MSFC composite score.

Administration of the MSFC begins with the T25FW to assess lower extremity physical function. Participants are told to “walk 25-feet as quickly, but as safely as possible” while being timed by the administrator. After repeating the T25FW once more, the 9HPT is administered to assess upper extremity physical function. Participants are presented with a peg board containing nine holes and a concave pocket holding 9 pegs. Starting with their dominant hand only, participants pick up each peg one at a time, place them in the holes, and when all the holes are filled, take each peg at one at a time and place them back in the container. This is repeated once more with the dominant hand, the board is switched for the non-dominant hand and repeated twice. See previous section for the administration of the SDMT. The MSFC score is computed by turning all participants’ scores into z scores. All three z scores are then averaged to create the composite z score number.

Imaging

Acquisition. All MS participants were scanned on a 3T Siemens Magnetom Skyra MRI Scanner. As part of a larger scanning session, the scans relevant to this study included a 3D gradient-echo T1-weighted sequences (MPRAGE, TR/TE/TI/flip angle = 2100ms/3.43ms/900ms/9 deg, resolution $1 \times 1 \times 1 \text{ mm}^3$, 256 mm FOV) and a diffusion tensor echo-planar-imaging (EPI) pulse sequence (TR/TE=9000ms/78ms, $2 \times 2 \times 2 \text{ mm}$ resolution, 256 FOV, with $b=0 \text{ s/mm}^2$ and $b=1100 \text{ s/mm}^2$). For preprocessing and analysis, DICOM files were converted to the NIFTI file format using MRICron dcm2nii software program (<https://www.nitrc.org/projects/dcm2nii/>).

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Volumetric preprocessing & analysis. FMRIB Software Library (FSL) version 5.0 (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) was used for all image preprocessing and analysis (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). FMRIB's Integrated Registration & Segmentation Tool (FIRST) was used to obtain volumes of left and right hippocampus as regions of interest (ROI) (Patenaude, Smith, Kennedy, & Jenkinson, 2011). Hippocampal masks were created using the Harvard atlas labels for the left and right hippocampus as a part of FSLeyes viewing program. Normalized Brain Volume, along with separate normalized grey and white matter volumes, were obtained using FSL's Structural Image Evaluation and Normalization of Atrophy for a single-time point (SIENAX) program, which normalizes volumes according to head and intracranial volume for each participant. Additionally, SIENAX outputs a Volume Scaling factor that is multiplied to the raw hippocampal volumes obtained from FIRST for normalization (S. M. Smith et al., 2004; S. M. Smith et al., 2002).

Voxel-wise analysis of hippocampal grey matter volume was investigated using FSL's Voxel-based Morphometry (VBM) (Douaud et al., 2007) according to the protocol by Good et al. (2002) utilizing various FSL tools (S. M. Smith et al., 2004). Structural images are first brain extracted and the grey matter is segmented before being registered to standard space with non-linear registration (Andersson, Jenkinson, & Smith, 2007). Resulting images are averaged and then flipped along the x-axis. Afterwards, all the grey matter images are non-linearly registered to a template created from the participants' data and corrected for irregularities due to spatial transformation. The images are finally smoothed using an isotropic Gaussian kernel with a sigma of 3 mm. Group analysis is then run using FSL's *randomise* paired *t*-test function on each of the left and right

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hippocampal ROIs with LDI scores as an EV to investigate the correlation between LDI scores and hippocampal voxels. The function uses a voxelwise permutation based (=5000) general linear model analysis with threshold-free cluster enhancement (TFCE) and correction for multiple comparisons.

Diffusion preprocessing. Diffusion weighted images were first eddy-current corrected and skull-stripped in FSL. The diffusion tensors are reconstructed using DTFIT. To obtain FA mean values and to perform voxel-wise statistical analysis of the FA data, FSL's TBSS was preformed (S. M. Smith et al., 2006). TBSS projects all participants' FA values onto a mean tract skeleton before applying voxel-wise statistics. Masks for white matter tracts connecting to and fro the hippocampus and subsections were created using John Hopkin's White Matter tract labels atlas and Juelich's Histological atlas as a part of FSLeves viewing software program. Group analyses and voxelwise statistics on imaging data were run using a general linear model and FSL's *randomise* paired *t*-test function. MS participants LDI scores were standardized using the mean and standard deviation LDI scores from a matched age group (Stark et al., 2013) and then split into memory groups based on MST LDI performance: MS participants at or below -0.5 standard deviations below the normative mean were considered impaired pattern separators (N=7) and any MS participants above -0.4 standard deviations were considered unimpaired (N=7). For the TBSS analysis, *randomise* was run with TFCE with 2D optimization to correct for multiple comparisons in order to investigate if any differences in FA exist between the MS groups.

Brain-Behavior Statistical Analysis

All statistical analyses between behavioral and imaging data were performed with the Statistical Package for Social Science (SPSS). Relationships between hippocampal volumes and behavioral and neuropsychological test scores were assessed using Pearson's r correlation coefficient, two-tailed tests with descriptive comparisons between measures of variance accounted for.

Results

Demographic, clinical, and neuropsychological characteristics of MS participants

Demographic and clinical characteristics of included MS participants ($n=14$) are presented in Table 1. The mean age of all participants is 39.14 years ($SD=4.11$), mean education is 15.43 years (1.60), and the mean Disease Duration is 6.36 years (2.37). There are 10 females and 4 males total. All participants had a Relapsing-remitting subtype.

Neuropsychological performance of the MS participants is shown in Table 2. Notably, MS participants' average score on all HVLT scores was below the normative mean. The average of all MS participants' scores on the BVMT were below the normative mean, except for the BVMT learning measure, which was at about average. Average performance on the DST is at the normative average. The MSFC composite score was slightly below normal and suggests moderate disease progression ($M=-0.40$).

Correlations between Brain Volumes and Behavioral & Neuropsychological tests

Correlations between volumes and test scores are presented in Table 3 and Figure 3 & 4. Voxel clusters surrounding the voxel with the highest significance value are presented in Table 4 for all significant imaging analyses.

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Normalized Brain Volume significantly correlated with all scores.

Overall cerebral grey matter volumes correlated significantly with the MST LDI scores ($r=.58$, $p<.05$) and the BVMT Delayed Recall score ($r=.60$, $p<.05$). The variance accounted for was similar between the two measures.

Overall cerebral white matter volumes significantly correlated with the MST LDI score ($r=.76$, $p<.01$), the BVMT Delayed Recall score ($r=.60$, $p<.05$), and the HVLT Delayed Recall score ($r=.53$, $p<.05$). The MST LDI score accounted for 22% more variance than the BVMT Delayed Recall score and 30% more variance than the HVLT Delayed Recall score.

Specific to the present hypothesis, left hippocampal volumes correlated significantly with the MST LDI ($r=.73$, $p<.01$) and the BVMT Delayed Recall score ($r=.56$, $p<.05$). The MST LDI score accounting for 22% more variance than the BVMT Delayed Recall score.

Right hippocampal volumes correlated significantly with MST LDI score ($r=0.72$, $p<.01$) and the BVMT Delayed Recall score ($r=.57$, $p<.05$) with the MST LDI score accounting for 19% more variance. No other correlations were significant (Table 3).

Brain by Voxel Volume Correlational Analysis

Results from the VBM analysis investigating the correlation between LDI scores and left hippocampal volume ROI (Figure 5) were significantly positively correlated (TFCE at or below $p=.05$). Results from the VBM analysis investigating the correlation between LDI scores and right hippocampal volume ROI (Figure 6) also revealed a significant positive correlation (TFCE at or below $p=.05$).

Diffusion parameters and Pattern Separation scores

The TBSS analysis comparing whole brain FA showed no significant differences in FA values between MS participants with high LDI scores and MS participants with low LDI scores. Further analysis focusing on white matter tracts connecting to the hippocampus, specifically the right and left cingulum and superior longitudinal fasciculus (temporal part only), also showed no significant differences between MS participants with high LDI scores and MS participants with low LDI scores.

General Discussion

At the onset of this study, pattern separation remained unexplored in studies of memory impairment in MS. Therefore, to investigate pattern separation in the MS population, we administered the MST (Stark et al., 2015; Stark et al., 2013) along with traditional neuropsychological measures for comparison. The main aims of this study were: (1) to evaluate the relationship between pattern separation performance and *a priori* ROI hippocampal volume, and (2) to investigate the relationship between pattern separation performance and microstructural integrity of *a priori* ROIs including white matter tracts connecting the hippocampus in MS. The data suggest the MST accounts for more variance than traditional neuropsychological assessments; but there were no observed relationships between the MST and white matter inputs to the hippocampus.

MS Hippocampal Volume and MST & Neuropsychological tests

Multiple volumetric analyses were run to investigate the relationship between MS hippocampal volume and pattern separation abilities. The first analysis involved extracting normalized hippocampal volumes from MS participants and comparing them to scores from the MST and traditional neuropsychological measure of memory to assess

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for correlational value. Additionally, correlations between pattern separation performance and voxels within the hippocampus of MS participants were assessed using VBM.

The results revealed significant positive correlations between pattern separation performance and normalized mean volumes of both the left and right hippocampi of MS participants. Specifically, lower LDI scores were associated with smaller hippocampal mean volumes. Additionally, significant correlations were observed between the BVMT Delayed Recall scores, a traditional neuropsychological measure of visuospatial memory, and both the left and right normalized hippocampi mean volumes. The nature of the MST and BVMT as visuospatial tests of memory support the role of the hippocampus in mediating the recall of visuospatial information. However, the LDI score correlations accounted for 22% more variance compared to the BVMT Delayed Recall score correlations in both left and right hippocampi, suggesting that measures of pattern separation may be more sensitive to hippocampal atrophy in MS. Further, this may suggest that the MST will be a valuable tool for clinicians to identify memory impairment for the clinician. The MST is a relatively quickly run test (~15 minutes with instructions) and is objectively scored. Tests like the BVMT are quick, but the nature of scoring the BVMT invites subjective input from scorers and thus creates inconsistency in results. Therefore, given the stronger relationship between pattern separation performance as assessed by the MST, the brevity of the MST, and the reliability of scoring, the MST may be a more useful tool to the clinical for assessing memory in MS than the BVMT.

These results corroborate similar results from a recent study published concurrently with the present research (Planche et al., 2017). In this study, persons with early MS, defined as someone who participated 6 to 18 months after their first neurologic

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episode suggestive of MS, and healthy controls both took the MST and BVMT. Results from this study showed no significant differences in any BVMT scores between the early MS and healthy control groups; however, there was a significant decrease in pattern separation performance in the early MS group compared to the healthy control group. The authors concluded that tests of pattern separation ability may be more sensitive to detecting early memory impairment in MS compared to the current traditional neuropsychological measures used (Planche et al., 2017). Our study's results support this finding by showing LDI scores account for more variance in both right and left hippocampal volumes of MS participants than the BVMT Delayed recall scores. However, the study by Planche et al. (2017) used persons with early MS and also compared them to healthy controls, and therefore more research is necessary to see how the onset of the disease affects any observed memory impairment in MS.

The present results also support past studies that suggest MS negatively affects hippocampal volume (Geurts et al., 2007; Rocca et al., 2015; Roosendaal et al., 2008), and that the hippocampal neuronal degeneration caused by MS is related to memory impairment (González Torre et al., 2017; Hulst et al., 2015; Kiy et al., 2011; Koenig et al., 2014; Muhlert et al., 2014; Planche et al., 2016; Sicotte et al., 2008; Sumowski et al., 2017; Sumowski et al., 2016). In this past research, traditional neuropsychological measures of memory were used to assess memory impairment in MS. For example, Sicotte et al. (2008) used the PASAT and a word list-learning task, and Koenig et al. (2014) used the BVMT-R, SDMT, PASAT, and the California Verbal Learning Test-II, a word list-learning task. The present study suggests that any robust study examining memory impairment in MS may be incomplete without tests of pattern separation.

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Clinical batteries using only traditional neuropsychological tests of memory may be missing aspects of memory that test of pattern separation are more sensitive to.

Therefore, clinical assessments including test of more aspects of memory, like pattern separation, may be better equipped to assess and develop treatments for their patients.

These findings could have additional implications for studies that did not observe memory impairment using only traditional neuropsychological measures despite finding hippocampal atrophy (Roosendaal et al., 2010). These studies may be making misleading or incomplete conclusions because of the aspect of memory left unassessed.

Assessing memory impairment in MS is a difficult process with major issues (Sandry, Akbar, et al., 2016). One major issue concerns what tests are used to assess memory impairment. Most studies of visuospatial memory in MS use the BMVT, or similar tests, as their sole assessment of this memory domain. The present results suggest that these assessments may be insensitive to the demands of patterns separation and to the associated hippocampal atrophy and, therefore, insufficient in assessing memory impairment in MS. When considering that this is one of the first studies to assess pattern separation in MS and the first to show a strong link to hippocampal function, there may be previous studies that concluded that memory impairment was evident in their MS sample; however, those investigations may not have exhaustively assessed subcomponents of memory acquisition. For example, one neuroimaging study used only the Location Learning Test to assess whether MS participants had intact memory function (Roosendaal et al., 2010). The authors then analyzed hippocampal volume differences between healthy controls and MS participants classified as having intact memory function solely based on the Location Learning Test. They found the MS group had

significantly smaller volumes in both their left and right hippocampus despite their classification of memory as behaviorally intact (Roosendaal et al., 2010). Results from the current study suggest that the authors may not have accurately and completely assessed visuospatial memory in their MS sample and that the hippocampal atrophy observed could be associated with unassessed memory impairments.

Other reasons that study conclusions are limited can be the use of composite scores, which may also mask differences in memory ability (Sandry, Akbar, et al., 2016). In one study, the authors used the SRT, a verbal list learning test, and the 10/36 Spatial Recall Test, a visuospatial memory test, to assess memory impairment in their sample (González Torre et al., 2017). The issues arise when the authors turn scores from both tests into z scores, combine them into a single composite score, and then create a cut-off score to classify which participants are exhibiting memory impairment. Most obviously, the creating of a single composite score of two tests assessing different domains, e.g. verbal and visual memory, precludes the authors from making any suggestions as to which specific tests or cognitive domains may be associated with the asymmetric hippocampal atrophy (see also, Sumowski et al., 2017). For instance, the authors cannot say whether the left hippocampal atrophy observed between groups is associated with verbal memory or visuospatial memory. The present study however did not use composite scores for the previously mentioned reasons. Avoiding composite scores allowed for the assessment between specific tests and brain volumes. Therefore, the use of composite scores limits any study's ability to specifically assess which tests are related to any other measures, especially measures of neuronal parameters.

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Further analysis investigated the correlation between pattern separation performance and voxels within the *a priori* defined left and right hippocampus. In-line with brain-behavior correlations, the results revealed voxel significant correlations between pattern separation performance and both the left and right hippocampus. Specifically, as volume increased, the ability to pattern separate also improved, which parallels our results showing correlations between normalized mean brain volumes of both the left and right hippocampus of MS participants and LDI scores. Additionally, VBM analyses using the HVLIT delayed recall scores and the BVMT recall scores as covariates revealed no significant correlations between volumes in either the left or right hippocampus. Taken together, the volumetric analyses performed in this study support pattern separation abilities being affected by MS-related hippocampal atrophy and suggest that assessments of pattern separation may be more sensitive measures of memory impairment and hippocampal atrophy in people with MS.

Diffusion Analysis

Initial tract-based statistical analysis comparing whole brain white matter tracts showed no significant differences in FA values between MS participants with low LDI scores and MS participants with high scores. Further TBSS analyses masked for left and right superior longitudinal fasciculus (temporal part) and cingulum, two tracts known to have connections to the hippocampus, also showed no significant differences between groups in FA values. The reasons why no significant differences were detected in hippocampal diffusion parameters between groups with differing pattern separation abilities could be due to the small sample size. This limitation is supported by a prior study using tract-based analysis to examine the white matter tracts of schizophrenic

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patients resampled and recreated different size subsets of the schizophrenic patients to assess whether the ability to detect changes in FA between groups depended on the size of the sample studied. The authors found a positive correlation between the sample size and the number of significant voxels reported, suggesting that a larger sample of MS participants may be needed to detect group differences in FA related to pattern separation ability (Melicher et al., 2015).

Past research has observed microstructural abnormalities affecting diffusion parameters in MS even when no atrophy or change in hippocampal volume was detected (Planche et al., 2016). Notably, the past study consisted of participants with clinically isolated syndrome, which means they had only one previous neurological episode related to MS. Due to the early stage of the disease duration, these participants might have had more variation in diffusion parameters, whereas the participants in the current study had an average disease duration of 6.36 years, well beyond the initial neurological episode. Therefore, it could be that microstructural integrity is affected first in MS but that at a certain point in disease duration, the point at which hippocampal atrophy manifests, microstructural variation may become more homogenous and hippocampal atrophy is a better measure of MS neurological insult especially as it relates to memory functioning. This assumption will need to be tested in future longitudinal research.

Implications Beyond the Current Study

The current study provides preliminary evidence that people with MS may have diminished pattern separation abilities. Additionally, the diminished pattern separation abilities correlate with hippocampal volume, and account for more variance in hippocampal volume than traditional neuropsychological measures of memory. Results

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corroborate Roll's theory stating that the hippocampus mediates pattern separation processes (Rolls, 2016; Treves & Rolls, 1992; Treves & Rolls, 1994). The theory states that pattern separation depends on interactions between the CA3 attractor network, mossy fiber input from the dentate gyrus, and perforant path input from the entorhinal cortex, while the CA1 subsection acts as the main input and output of the hippocampal pattern separation system (Treves & Rolls, 1994). Therefore, a loss in volume in these areas will likely impair pattern separation performance, and this was observed in the current study of pattern separation performance in a sample of MS participants. The next step in analysis is to further segment the hippocampus into its subsections to assess what specific parts of the hippocampus undergo MS-related atrophy. If studies assessing pattern separation ability in MS find associations between the volume of the CA3 and dentate gyrus subsections of the hippocampus specifically, then Roll's theory would be further supported in MS samples (Rolls, 2016).

Together, at most, the results suggest that pattern separation assessments should be a part of the clinician's battery of tests used to assess memory impairment. At the least, these results provide evidence for future research to further investigate how pattern separation abilities may be affected by the neurodegenerative effects of MS, ideally in larger samples. Moreover, if tests of pattern separation are more sensitive to cognitive impairment in MS, they could potentially quicken the recognition and diagnosis and also provide different avenues of treatment for MS-related memory impairment. Neurogenesis is one potential avenue of treatment that could be looked at for pattern separation deficits.

The potential treatment lies in the affect exercise has on neurogenesis. An abundance of research suggests adult neurogenesis occurs in the hippocampus

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(Kempermann, Song, & Gage, 2015; Zhao, Deng, & Gage, 2008) although there are other regions in the brain that produce new neurons (Lazarini et al., 2014). Neurogenesis in the dentate gyrus has been linked to memory formation, consolidation, and retrieval (Gu et al., 2012; Kitamura et al., 2009; Shors et al., 2001). Adult hippocampal neurogenesis generates new granule cells in the dentate gyrus, an area critical for proper pattern separation performance, and this suggests a potential target for treatment (Kempermann et al., 2015; Rolls & Kesner, 2016). Exercise is one activity that directly affects neurogenesis. Research in both animals and humans shows long-term, aerobic exercise to increase adult neurogenesis and improve memory (Speisman, Kumar, Rani, Foster, & Ormerod, 2013; Voss, Vivar, Kramer, & van Praag, 2013). Perhaps aerobic exercise may be able to provide treatment to the population of MS experiencing cognitive impairment but retain ambulatory ability. The MST may be an appropriate primary outcome for future exercise trials in MS.

A preliminary study examining the effects of aerobic exercise on hippocampal function and connectivity and memory performance in MS found a large increase in memory performance related to increases in hippocampal volume and functional connectivity (Leavitt et al., 2014). A recent pilot study aimed to examine the effect exercise may have on the hippocampus of MS participants (Sandroff, Johnson, & Motl, 2017). The authors assessed the baseline learning and memory status of MS participants using the California Verbal Learning Test II and ran a baseline imaging scan on MS participants at the beginning of the study. The scan included a non-conventional neuroimaging method assessing viscoelasticity of the hippocampus; viscoelasticity has been associated with the function of the hippocampus, in that lower tissue viscosity was

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associated with better memory (Schwarb, Johnson, McGarry, & Cohen, 2016). After baseline tests and scans, the MS participants were split into control and treatment groups. The treatment group underwent 12 weeks of treadmill exercising while the control group did not and were asked not to undertake any additional exercise outside of their normal routine (Sandroff et al., 2017). After the 12 weeks, both groups were re-scanned and re-assessed on the learning and memory measure. The authors found that the exercise group showed a moderate increase in learning and memory scores compared to the non-exercise group. Additionally, the results showed a strong relationship between the improvement of learning and memory performance and viscoelasticity of the hippocampus (Sandroff et al., 2017). Notably, however, this study was a pilot and contained only 8 MS participants and no other measures of memory. Additional research specifically investigating the effect of exercise on impaired pattern separation has showed promising results (Ryan & Nolan, 2016; Sahay et al., 2011). Therefore, the clinical implications of assessing pattern separation potentially extend beyond assessment into treatment development.

Another clinical implication of the current study's results concerns the comorbidity of depression and MS. Depression occurs in about 40-50% of people with MS (Feinstein, Magalhaes, Richard, Audet, & Moore, 2014; Patten, Marrie, & Carta, 2017). Although the etiology of depression in MS is likely not due to a single factor, depression in MS has been associated with temporal regions (Berg et al., 2000; Zivadinov et al., 2001) and with the hippocampus specifically (Gold et al., 2010; Gold et al., 2014; Kiy et al., 2011). One study segmented the subsections of the hippocampus and the surrounding areas to assess whether volumes of any regions correlated with depression scores via the Beck Depression Inventory (Gold et al., 2010). The authors found that the

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MS participants with depressive symptoms showed smaller CA3 and dentate gyrus volumes as well as higher cortisol levels compared to MS participants with no depressive symptoms (Gold et al., 2010). Another study using volumetric and shape analyses examined the relationship between depression and the hippocampus in MS. Using the Center for Epidemiologic Studies—Depression scale to assess depression, the authors found that MS participants with high levels of depression showed atrophy and shape changes in the right hippocampus when compared to the non-depressed MS participants (Gold et al., 2014). To investigate the relationship between depression and pattern separation performance in a healthy population, another study administered a pattern separation test similar to the MST and used questionnaires to assess depression and observed a significant negative relationship (Shelton & Kirwan, 2013). Future research should directly investigate the relationship between depression and pattern separation abilities in MS.

Interestingly, in animal models, antidepressant drug treatments can also increase neurogenesis and neuroplasticity in the hippocampus (Kugathasan et al., 2017; Li et al., 2015; Malberg, Eisch, Nestler, & Duman, 2000). Furthermore, a combination of exercise and antidepressant treatment increases brain-derived neurotrophic factor, a chemical strongly associated with neurogenesis, in healthy and aging rat samples (Garza, Ha, Garcia, Chen, & Russo-Neustadt, 2004; Russo-Neustadt, Beard, Huang, & Cotman, 2000). Therefore, if research continues to observe a relationship between hippocampal integrity and pattern separation memory performance, a future study could focus on the treatment effects of antidepressants and/or exercise on memory impairment in MS.

Limitations

The current study is not without limitations. The main limitation of this study is the small sample size and limited statistical power. The sample size makes it difficult to make any definitive conclusions about the findings but does not negate the need for future research into pattern separation abilities of MS participants. Another limitation is the lack of a control group. Although we are aware of the structural and microstructural neuronal differences between the healthy population and the MS population, a healthy sample for comparison is still important to determine whether MS pattern separation abilities significantly differ from individuals without MS. A recent study mentioned previously suggests that MS participants experience pattern separation impairments when compared to healthy controls (Planche et al., 2017) and our sample of MS did score below the age-related normative mean as outlined by Stark et al. (2013).

Another limitation is that the analysis did not control for by age, sex, education, or disease duration in this small pilot study. This is a limitation because these factors could be contributing to the observed results. As one of the first studies assessing pattern separation ability in MS, this study was intended to serve as a descriptive purpose as to whether future studies should continue in this direction. In that capacity, this study has succeeded in providing enough evidence to suggest that more studies into how pattern separation ability is affected by MS and how assessments of pattern separation in MS could serve as more sensitive markers of hippocampal atrophy or abnormality.

A limitation exists concerning the diffusion image processing. Diffusion imaging is performed using diffusion weighted spin-echo EPI, which is sensitive to non-zero resonance fields. These fields can be caused by the nature of the participant's head,

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known as the susceptibility-induced off-resonance field, and by eddy currents created from the rapid switching of gradients during the diffusion weighted scan (S. M. Smith et al., 2004). The susceptibility-induced off-resonance field is held constant for all acquired images which causes a geometric mismatch between the diffusion images and the structural images. Topup is an FSL tool that corrects for this mismatch. At the time of analysis, we were unable to correct for mismatch using Topup because scan sequence only contained a single direction, $b=0$.

Another issue worth mentioning concerns how lower level input can affect higher order cognitive functioning. Optic neuritis is frequent amongst people with MS (Malik et al., 2014). Given the visual nature of the MST, this could have affected scores by providing inaccurate or incomplete input to the hippocampus. However, if lower-level (cortically speaking) visual input was causing lower LDI scores observed in this experiment, then we could expect to see similar results for the MST recognition scores, which we do not.

Conclusion

This study provides initial evidence that MS neuropathology may adversely affect pattern separation ability, and that this impairment could be related to a decrease in hippocampal volume. Further, the results suggest that tests of pattern separation may be more sensitive to memory impairment and accompanying MS-induced hippocampal atrophy. Future research should assess pattern separation ability and its relation to hippocampal subsection volume and microstructural integrity in larger samples of MS. Potential implications of the results extend into clinical assessments and treatment of MS.

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Table 1

MS Demographics and Clinical Characteristics

Demographics	MS Participants (14)
Mean Age (SD)	39.14 (4.11)
Sex Ratio (F/M)	10/4
Mean Education, years (SD)	15.43 (1.60)
Mean Disease Duration, years (SD)	6.36 (2.37)

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Table 2

MS Neuropsychological Performance

Test	Average	SD
SDMT	53.06	16.59
WTAR	33.13	9.46
WTAR Scaled Score	99.47	14.14
HVLT-R Trial 1	5.94	1.65
HVLT-R Trial 1 T-score	40.69	9.66
HVLT-R Total Recall	24.56	4.44
HVLT-R Total Recall T-score	41.50	10.60
HVLT-R Delayed Recall	8.25	2.11
HVLT-R Delayed Recall T-score	41.13	10.22
Digit Span Forward	9.31	1.54
Digit Span Forward Scaled Score	8.44	1.71
Digit Span Backwards	8.25	1.44
Digit Span Backwards Scaled Score	9.25	1.44
Digit Span Sequencing	7.63	2.60
Digit Span Sequencing Scaled Score	8.44	1.71
Digit Span Total	25.19	3.39
Digit Span Total Scaled Score	8.44	1.82
BVMT-R Trial 1	4.75	2.52
BVMT-R Trial 1 T-score	41.75	12.38
BVMT-R Trial 2	7.56	2.90
BVMT-R Trial 2 T-score	42.63	14.09
BVMT-R Trial 3	8.94	2.89
BVMT-R Trial 3 T-score	44.25	15.08
BVMT-R Total Score	21.19	7.30
BVMT-R Total Score T-score	41.25	13.27
BVMT-R Delayed Score	8.50	2.92
BVMT-R Delayed Score T-score	44.38	13.91
BVMT-R Learning	4.31	2.41
BVMT-R Learning T-score	52.69	15.58
BVMT-R Percent Retained	95%	25%
BVMT-R Recognition Discrimination Index	5.56	0.73
MSFC Composite Score	-0.40	0.94

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Table 3

Correlations between Brain Volumes and Behavioral & Neuropsychological tests

	Normalized Brain Volume	Grey Matter Volume	White Matter Volume	Left Hippocampus	Right Hippocampus	MST, LDI	MST, Recognition	HVLT, Delayed Recall	HVLT, RDI	BVMT, Delayed Recall
Normalized Brain Volume	1									
Grey Matter Volume	.91**	1								
White Matter Volume	.87**	.58*	1							
Left Hippocampus	.91**	.74**	.68**	1						
Right Hippocampus	.87**	.63*	.43	.81**	1					
MST, LDI	.80**	.58*	.76**	.73**	.72**	1				
MST, Recognition	.87**	.34	.39	.46	.34	.35	1			
HVLT, Delayed Recall	.80**	.44	.53*	.27	.33	.51	.56*	1		
HVLT, RDI	.61*	.35	.29	.43	.47	.33	.82**	.37	1	
BVMT, Delayed Recall	.80**	.60*	.60*	.56*	.57*	.57*	.76**	.65*	.73**	1

** . Correlation is significant at the .01 level (2-tailed).

* . Correlation is significant at the

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.05 level (2-tailed).

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Table 4

Clusters of Voxels surrounding the Voxel with Max p value

Analysis	Voxels	<i>p</i> value	Max X (mm)	Max Y (mm)	Max Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)
Left Hippocampal Volume Difference	153	0.05	-16	-36	-2	-17.9	-32.3	-5.55
LDI Correlation, Left Hippocampus	367	0.05	-16	-38	0	-22.1	-32.9	-5.63
LDI Correlation, Right Hippocampus	534	0.05	20	-22	-12	27.2	-27.4	-8.81

Notes. COG = Center of Gravity

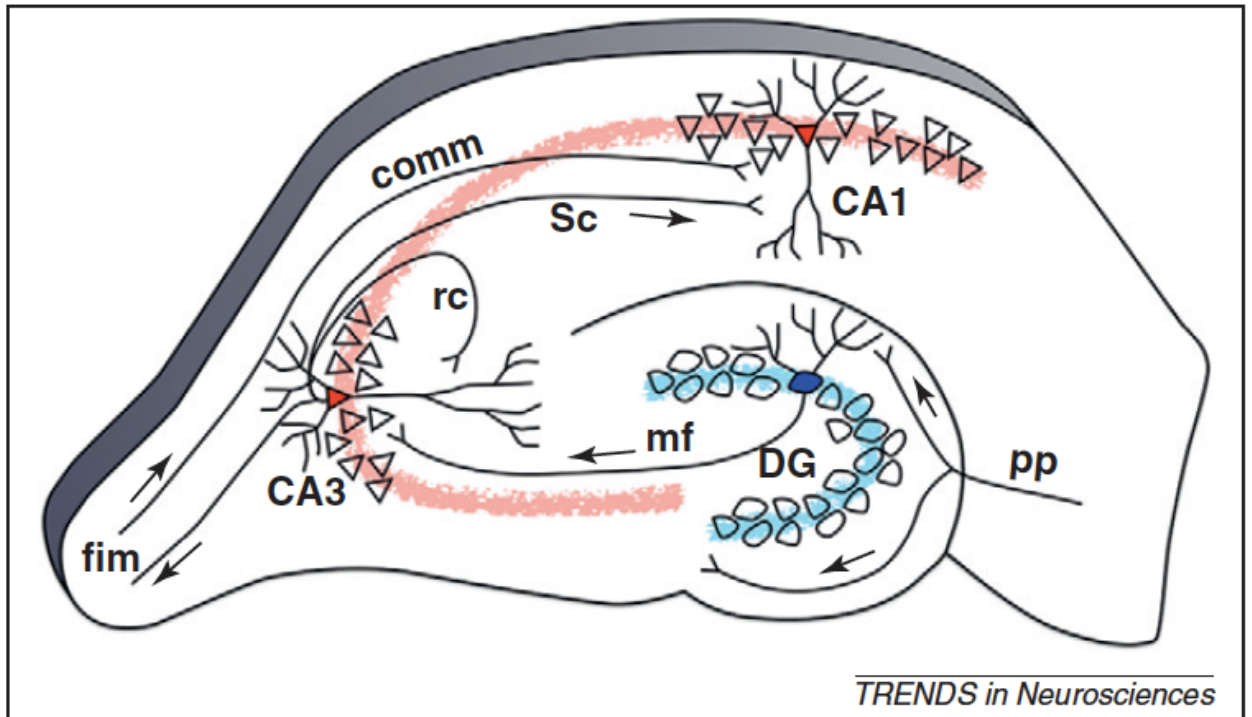


Figure 1. Rat Hippocampus showing CA3 inputs.

Coronal slice of the rat hippocampus showing the cornu ammonis (CA) subsections, dentate gyrus (DG), mossy fibers (mf), perforant pathway (pp), and recurrent collaterals (rc). The CA3 subregion contains recurrent collaterals, which are neurons that synapse on themselves or other neurons within the same network. The CA3 subsection receives input from mossy fiber cells in the dentate gyrus and from layer II cells originating in the entorhinal cortex (not pictured) and extending to CA3 using the perforant pathway.

Figure adapted from Yassa and Stark (2011).

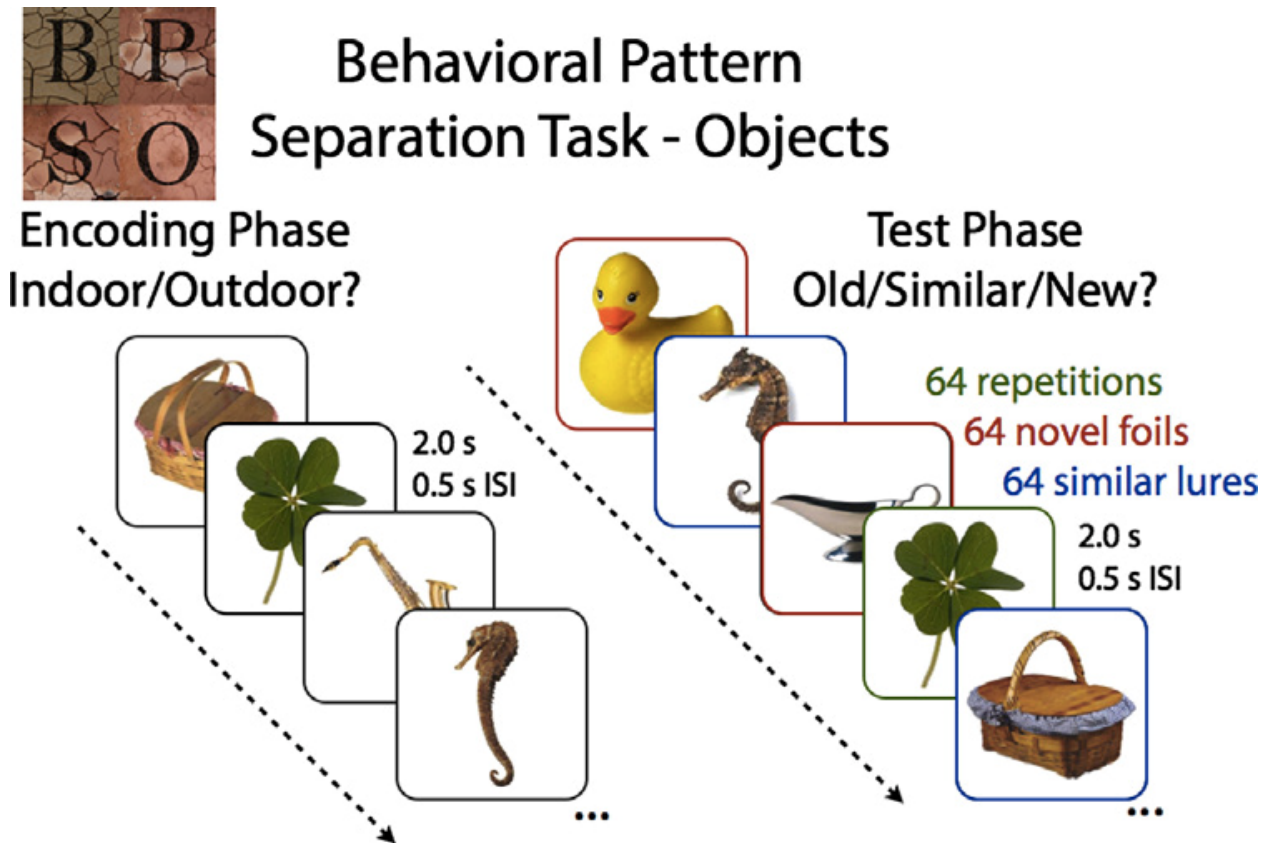


Figure 2. MST Task.

In the Mnemonic Similarities Test (formally known as the Behavioral Pattern Separation Task) participants incidentally encoded a series of pictures followed by a surprise recognition test, which consisted of old items (repetitions), similar items (lures), and completely new items (novel foils) (Stark et al., 2013).

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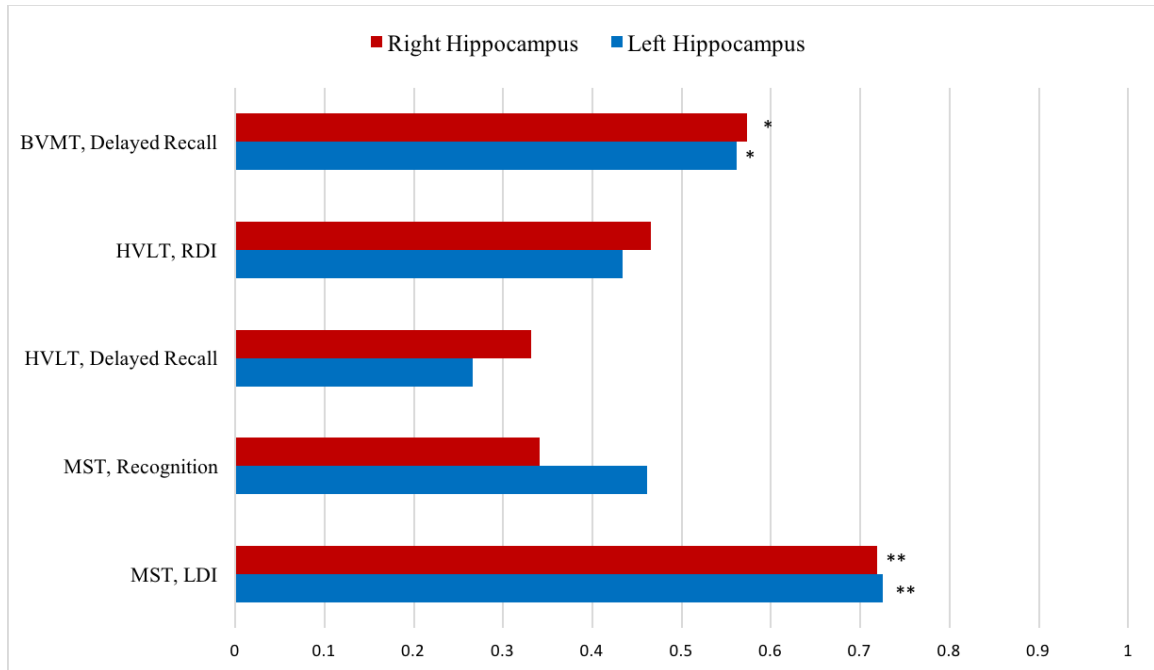


Figure 3. Hippocampal Volume Correlations with MST and neuropsychological tests. Correlations between Mnemonic Similarities Test scores, traditional neuropsychological measures of memory and left & right hippocampal volumes (* is $p < .10$, and ** means $p < 0.05$).

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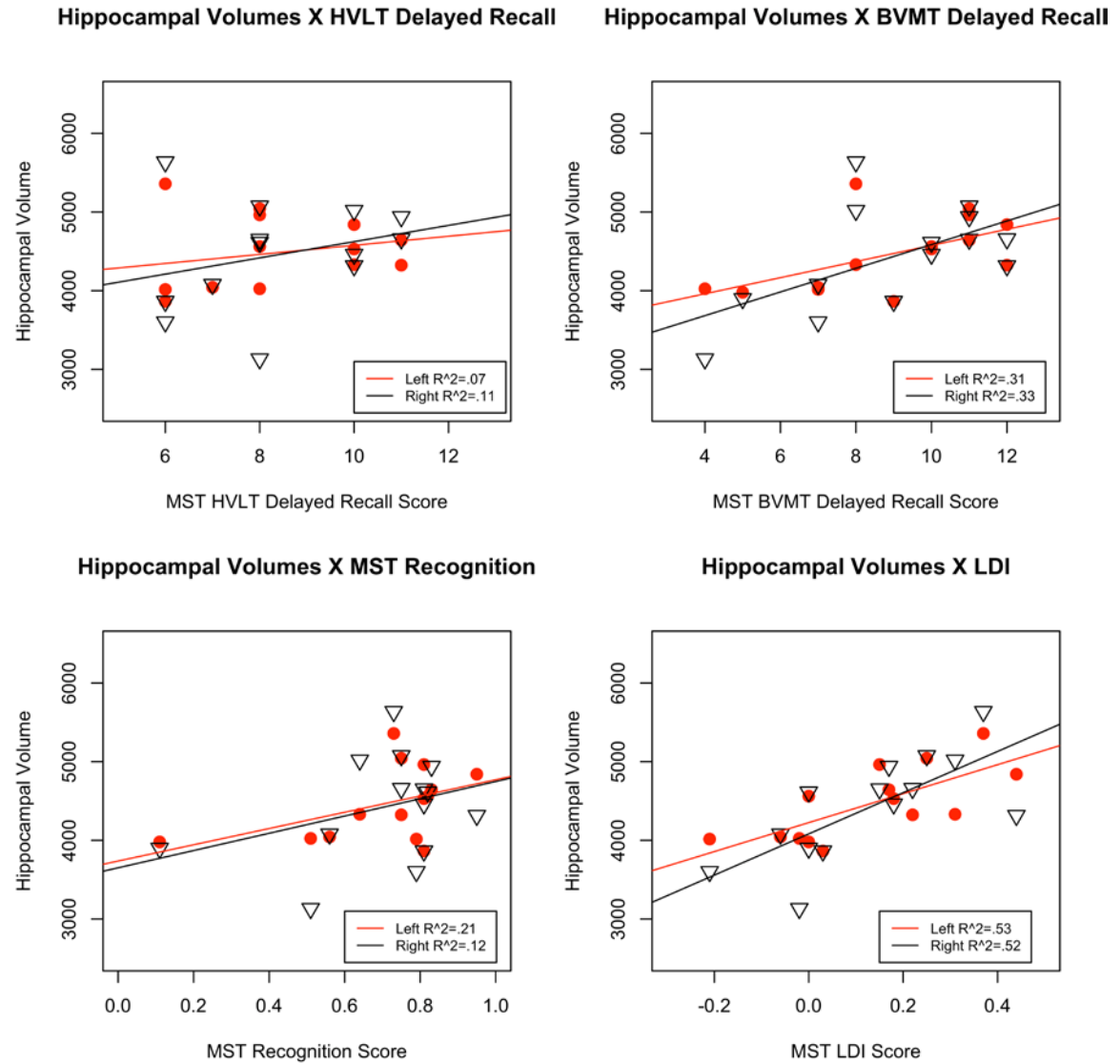


Figure 4. Correlations.

Correlations between test scores and left & right hippocampal volumes. The left side shows non-significant correlations between hippocampal volumes and $p=.05$ HVL Delayed recall scores (top left) and MST Recognition scores (bottom left). The right side shows significant correlations at $p=.05$ between hippocampal volumes and BVMT Delayed Recall scores (top right) and MST LDI Scores (bottom right).

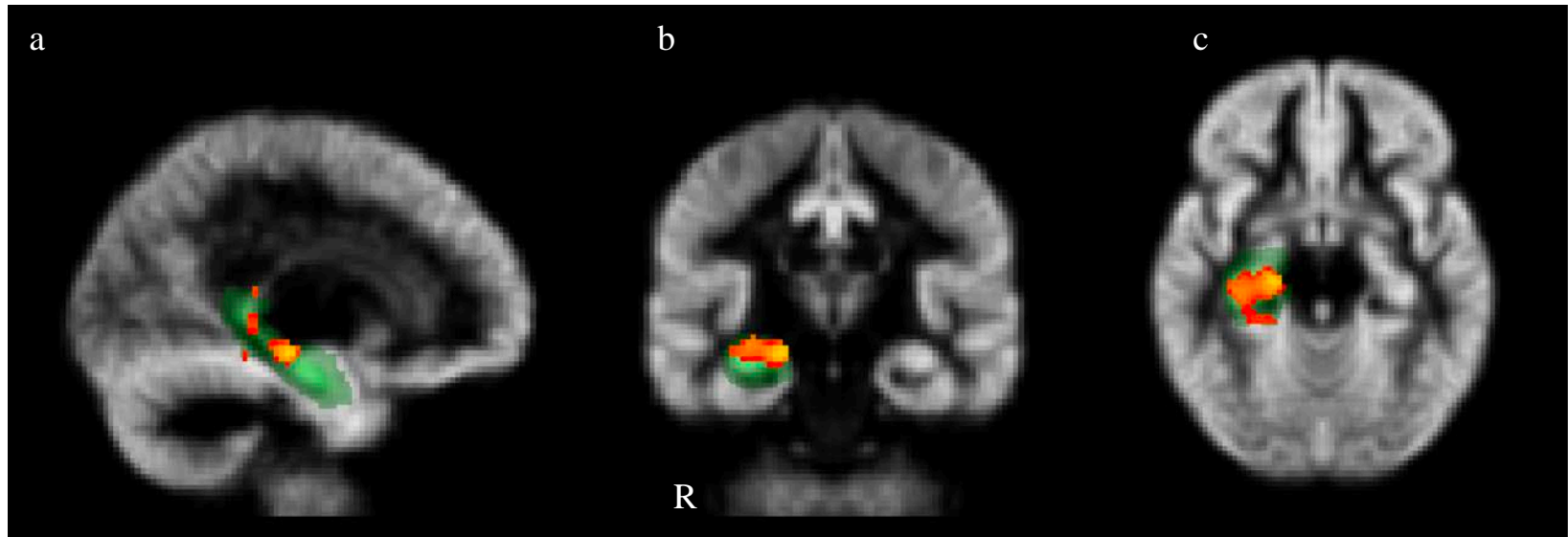


Figure 5. Right Hippocampal Voxels correlating with LDI scores.

Results of the Voxel-based Morphometry analysis investigating the correlation between LDI scores and volume of the right hippocampus shown in sagittal (a) coronal (b) and horizontal (c) slices. The opaque green represents Harvard's atlas of the right hippocampus taken from FSLeyes atlases. Red to yellow heat map shows areas of significant correlation between right hippocampal volume and LDI scores at $p < .05$ significance level.

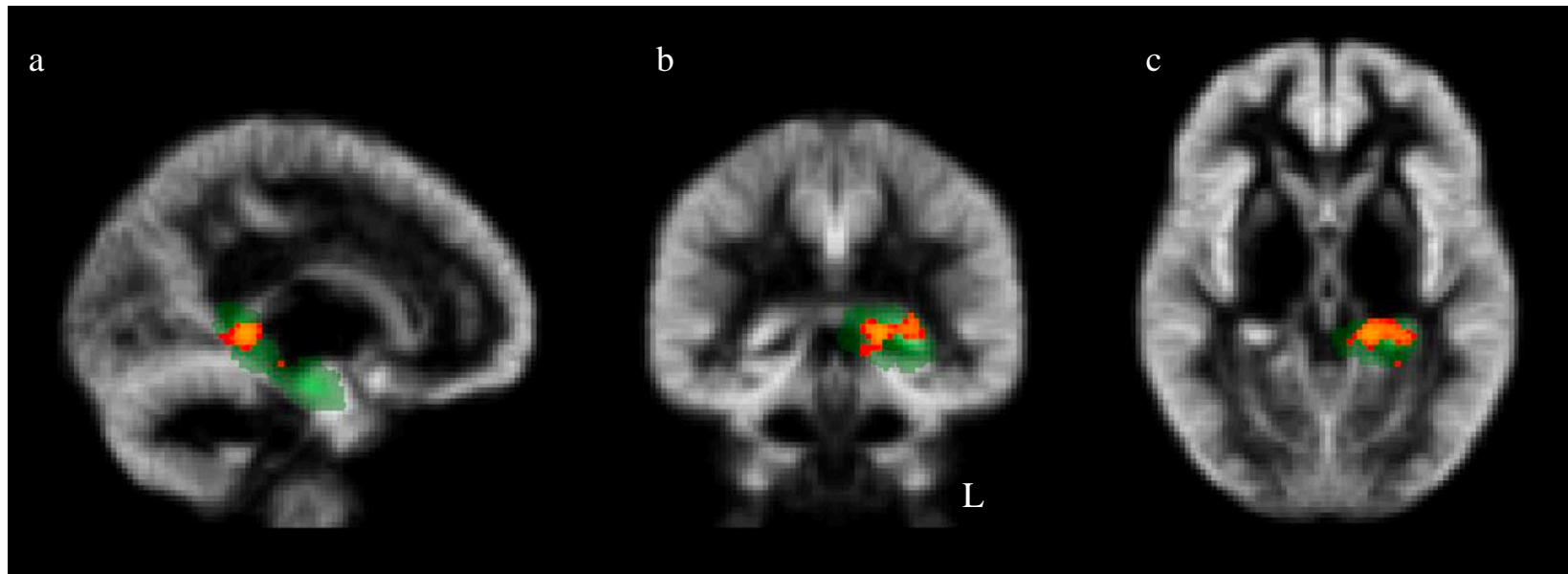


Figure 6. Left Hippocampal Volumes correlating with LDI scores.

Results of the Voxel-based Morphometry analysis investigating the correlation between LDI scores and volume of the left hippocampus shown in sagittal (a) coronal (b) and horizontal (c) slices. The opaque green represents Harvard's atlas of the left hippocampus taken from FSLeyes atlases. Red to yellow heat map shows areas of significant correlation between left hippocampal volume and LDI scores at $p < .05$ significance level.